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(54) Title: ANTISENSE MODULATION OF G-ALPHA-i3 EXPRESSION

(57) Abstract: Antisense compounds, compositions and methods are provided for modulating the expression of G-alpha-i3. The compositions comprise antisense compounds, particularly antisense oligonucleotides, targeted to nucleic acids encoding G-alpha-i3. Methods of using these compounds for modulation of G-alpha-i3 expression and for treatment of diseases associated with expression of G-alpha-i3 are provided.

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ANTISENSE MODULATION OF G-ALPHA-i3 EXPRESSION

FIELD OF THE INVENTION

The present invention provides compositions and
5   methods for modulating the expression of G-alpha-i3. In
particular, this invention relates to antisense compounds,
particularly oligonucleotides, specifically hybridizable
with nucleic acids encoding human G-alpha-i3. Such
oligonucleotides have been shown to modulate the expression
10   of G-alpha-i3.

BACKGROUND OF THE INVENTION

A vast majority of biologically active molecules
including growth factors, cytokines, neurotransmitters and
hormones transduce signals via specific cell-surface
15   receptors. Some of these receptors are then coupled to
heterotrimeric GTP-binding proteins (G proteins) which,
upon activation, relay signals to a variety of cellular
effectors including at least four phospholipase C (PLC)
variants and adenylyl cyclases.

20   G proteins mediate external signals by forming
heterotrimers consisting of an alpha, beta and gamma
subunit. Several isoforms of each subunit have been
identified and therefore, through subunit heterogeneity, G
proteins effectively integrate multiple signaling cascades.
25   The alpha subunits of G proteins contain the GTP binding
site and intrinsic catalytic GTPase activity. Based on
sequence similarity and function, these subunits have been
classified into four major groups; Gs, which stimulate
adenylyl cyclases; Gi, which inhibit adenylyl cyclases; Gq,
30   which activate PLC isoforms and G12/13, which mediate
pathways associated with cell growth and differentiation
(Hamm, *J. Biol. Chem.*, *1998*, *273*, 669-672).

G-alpha-i3 is a member of the Gi subfamily of G
proteins which is involved in hormonal inhibition of
35   adenylyl cyclase and in the regulation of plasma membrane

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enzymes. G-alpha-i3 has also been shown to mediate dopamine, thyrotropin-releasing hormone and somatostatin signal transduction pathways (Kineman et al., *Endocrinology*, **1994**, *135*, 790-793; Kineman et al., *J.*

5 *Endocrinol.*, **1996**, *148*, 447-455; Law et al., *J. Biol. Chem.*, **1993**, *268*, 10721-10727). In addition, an upstream inhibitor of phospholipase C, U73122, resulted in the inhibition of Gi-mediated protein activation further implicating G-alpha-i3 in lipid signaling cascades (Wu et

10 al., *Neuroreport.*, **1998**, *9*, 99-103).

Comparison studies of modified forms of the pertussis toxin-insensitive form of G-alpha-i3 (pertussis toxin is normally an inhibitor of Gi function) and the wild type protein, in assays designed to investigate the interactions

15 of adrenoceptors and Gi proteins, demonstrated that more agonist was required to stimulate the mutant protein than the wild type. These studies showed that the affinity of the wild type Gi protein for the receptor was greater than that of the mutant (Wise et al., *Biochem. J.*, **1997**, *321*,

20 721-728).

In human hepatocellular carcinoma (HCC), the expression and functional activity of G-alpha-i3 was increased in 80% of the tumors examined. These results indicate that the regulation of the adenylate cyclase

25 system in these cells may contribute to the formation or progression of the carcinoma (Schmidt et al., *Hepatology*, **1997**, *26*, 1189-1194).

The expression of G-alpha-i3 has also been shown to be regulated by physiologic shear stresses such as flow.

30 Using a transcapillary coculture system, it was shown that G-alpha-i3 expression was increased by high-flow conditions in endothelial and vascular smooth muscle cells implicating Gi proteins in flow-induced responses of vessel wall

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function (Redmond et al., *Arterioscler. Thromb. Vasc. Biol.*, 1998, *18*, 75-83).

Currently, there are no known therapeutic agents which effectively inhibit the synthesis of G-alpha-i3 and to date strategies aimed at inhibiting or investigating G-alpha-i3 function have predominantly involved the use of antibodies.

These strategies, however, are untested as therapeutic protocols. Consequently there remains a long felt need for additional agents capable of effectively inhibiting G-alpha-i3 function and antisense oligonucleotides may provide a promising new pharmaceutical tool for the effective and specific modulation of G-alpha-i3 expression.

SUMMARY OF THE INVENTION

The present invention is directed to antisense compounds, particularly oligonucleotides, which are targeted to a nucleic acid encoding G-alpha-i3, and which modulate the expression of G-alpha-i3. Pharmaceutical and other compositions comprising the antisense compounds of the invention are also provided. Further provided are methods of modulating the expression of G-alpha-i3 in cells or tissues comprising contacting said cells or tissues with one or more of the antisense compounds or compositions of the invention. Further provided are methods of treating an animal, particularly a human, suspected of having or being prone to a disease or condition associated with expression of G-alpha-i3 by administering a therapeutically or prophylactically effective amount of one or more of the antisense compounds or compositions of the invention.

DETAILED DESCRIPTION OF THE INVENTION

The present invention employs oligomeric antisense compounds, particularly oligonucleotides, for use in modulating the function of nucleic acid molecules encoding G-alpha-i3, ultimately modulating the amount of G-alpha-i3 produced. This is accomplished by providing antisense compounds which specifically hybridize with one or more

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nucleic acids encoding G-alpha-i3. As used herein, the terms "target nucleic acid" and "nucleic acid encoding G-alpha-i3" encompass DNA encoding G-alpha-i3, RNA (including pre-mRNA and mRNA) transcribed from such DNA, and also cDNA derived from such RNA. The specific hybridization of an oligomeric compound with its target nucleic acid interferes with the normal function of the nucleic acid. This modulation of function of a target nucleic acid by compounds which specifically hybridize to it is generally referred to as "antisense". The functions of DNA to be interfered with include replication and transcription. The functions of RNA to be interfered with include all vital functions such as, for example, translocation of the RNA to the site of protein translation, translation of protein from the RNA, splicing of the RNA to yield one or more mRNA species, and catalytic activity which may be engaged in or facilitated by the RNA. The overall effect of such interference with target nucleic acid function is modulation of the expression of G-alpha-i3. In the context of the present invention, "modulation" means either an increase (stimulation) or a decrease (inhibition) in the expression of a gene. In the context of the present invention, inhibition is the preferred form of modulation of gene expression and mRNA is a preferred target.

It is preferred to target specific nucleic acids for antisense. "Targeting" an antisense compound to a particular nucleic acid, in the context of this invention, is a multistep process. The process usually begins with the identification of a nucleic acid sequence whose function is to be modulated. This may be, for example, a cellular gene (or mRNA transcribed from the gene) whose expression is associated with a particular disorder or disease state, or a nucleic acid molecule from an infectious agent. In the present invention, the target is a nucleic acid molecule encoding G-alpha-i3. The targeting

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process also includes determination of a site or sites
within this gene for the antisense interaction to occur
such that the desired effect, e.g., detection or modulation
of expression of the protein, will result. Within the
5   context of the present invention, a preferred intragenic
site is the region encompassing the translation initiation
or termination codon of the open reading frame (ORF) of the
gene. Since, as is known in the art, the translation
initiation codon is typically 5'-AUG (in transcribed mRNA
10 molecules; 5'-ATG in the corresponding DNA molecule), the
translation initiation codon is also referred to as the
"AUG codon," the "start codon" or the "AUG start codon". A
minority of genes have a translation initiation codon
having the RNA sequence 5'-GUG, 5'-UUG or 5'-CUG, and
15 5'-AUA, 5'-ACG and 5'-CUG have been shown to function in
vivo. Thus, the terms "translation initiation codon" and
"start codon" can encompass many codon sequences, even
though the initiator amino acid in each instance is
typically methionine (in eukaryotes) or formylmethionine
20 (in prokaryotes). It is also known in the art that
eukaryotic and prokaryotic genes may have two or more
alternative start codons, any one of which may be
preferentially utilized for translation initiation in a
particular cell type or tissue, or under a particular set
25 of conditions. In the context of the invention, "start
codon" and "translation initiation codon" refer to the
codon or codons that are used in vivo to initiate
translation of an mRNA molecule transcribed from a gene
encoding G-alpha-i3, regardless of the sequence(s) of such
30 codons.

It is also known in the art that a translation
termination codon (or "stop codon") of a gene may have one
of three sequences, i.e., 5'-UAA, 5'-UAG and 5'-UGA (the
corresponding DNA sequences are 5'-TAA, 5'-TAG and 5'-TGA,
35 respectively). The terms "start codon region" and

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"translation initiation codon region" refer to a portion of such an mRNA or gene that encompasses from about 25 to about 50 contiguous nucleotides in either direction (i.e., 5' or 3') from a translation initiation codon. Similarly,

5   the terms "stop codon region" and "translation termination codon region" refer to a portion of such an mRNA or gene that encompasses from about 25 to about 50 contiguous nucleotides in either direction (i.e., 5' or 3') from a translation termination codon.

10   The open reading frame (ORF) or "coding region," which is known in the art to refer to the region between the translation initiation codon and the translation termination codon, is also a region which may be targeted effectively. Other target regions include the 5'

15   untranslated region (5'UTR), known in the art to refer to the portion of an mRNA in the 5' direction from the translation initiation codon, and thus including nucleotides between the 5' cap site and the translation initiation codon of an mRNA or corresponding nucleotides on

20   the gene, and the 3' untranslated region (3'UTR), known in the art to refer to the portion of an mRNA in the 3' direction from the translation termination codon, and thus including nucleotides between the translation termination codon and 3' end of an mRNA or corresponding nucleotides on

25   the gene. The 5' cap of an mRNA comprises an N7-methylated guanosine residue joined to the 5'-most residue of the mRNA via a 5'-5' triphosphate linkage. The 5' cap region of an mRNA is considered to include the 5' cap structure itself as well as the first 50 nucleotides adjacent to the cap.

30   The 5' cap region may also be a preferred target region.

        Although some eukaryotic mRNA transcripts are directly translated, many contain one or more regions, known as "introns," which are excised from a transcript before it is translated. The remaining (and therefore translated)

35   regions are known as "exons" and are spliced together to

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form a continuous mRNA sequence. mRNA splice sites, i.e.,
intron-exon junctions, may also be preferred target
regions, and are particularly useful in situations where
aberrant splicing is implicated in disease, or where an
5   overproduction of a particular mRNA splice product is
implicated in disease. Aberrant fusion junctions due to
rearrangements or deletions are also preferred targets. It
has also been found that introns can also be effective, and
therefore preferred, target regions for antisense compounds
10   targeted, for example, to DNA or pre-mRNA.

Once one or more target sites have been identified,
oligonucleotides are chosen which are sufficiently
complementary to the target, i.e., hybridize sufficiently
well and with sufficient specificity, to give the desired
15   effect.

In the context of this invention, "hybridization"
means hydrogen bonding, which may be Watson-Crick,
Hoogsteen or reversed Hoogsteen hydrogen bonding, between
complementary nucleoside or nucleotide bases. For example,
20   adenine and thymine are complementary nucleobases which
pair through the formation of hydrogen bonds.
"Complementary," as used herein, refers to the capacity for
precise pairing between two nucleotides. For example, if a
nucleotide at a certain position of an oligonucleotide is
25   capable of hydrogen bonding with a nucleotide at the same
position of a DNA or RNA molecule, then the oligonucleotide
and the DNA or RNA are considered to be complementary to
each other at that position. The oligonucleotide and the
DNA or RNA are complementary to each other when a
30   sufficient number of corresponding positions in each
molecule are occupied by nucleotides which can hydrogen
bond with each other. Thus, "specifically hybridizable"
and "complementary" are terms which are used to indicate a
sufficient degree of complementarity or precise pairing
35   such that stable and specific binding occurs between the

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oligonucleotide and the DNA or RNA target. It is
understood in the art that the sequence of an antisense
compound need not be 100% complementary to that of its
target nucleic acid to be specifically hybridizable. An
5   antisense compound is specifically hybridizable when
binding of the compound to the target DNA or RNA molecule
interferes with the normal function of the target DNA or
RNA to cause a loss of utility, and there is a sufficient
degree of complementarity to avoid non-specific binding of
10 the antisense compound to non-target sequences under
conditions in which specific binding is desired, i.e.,
under physiological conditions in the case of in vivo
assays or therapeutic treatment, and in the case of in
vitro assays, under conditions in which the assays are
15 performed.

Antisense compounds are commonly used as research
reagents and diagnostics. For example, antisense
oligonucleotides, which are able to inhibit gene expression
with exquisite specificity, are often used by those of
20 ordinary skill to elucidate the function of particular
genes. Antisense compounds are also used, for example, to
distinguish between functions of various members of a
biological pathway. Antisense modulation has, therefore,
been harnessed for research use.

25   The specificity and sensitivity of antisense is also
harnessed by those of skill in the art for therapeutic
uses. Antisense oligonucleotides have been employed as
therapeutic moieties in the treatment of disease states in
animals and man. Antisense oligonucleotides have been
30 safely and effectively administered to humans and numerous
clinical trials are presently underway. It is thus
established that oligonucleotides can be useful therapeutic
modalities that can be configured to be useful in treatment
regimes for treatment of cells, tissues and animals,
35 especially humans.

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In the context of this invention, the term
"oligonucleotide" refers to an oligomer or polymer of
ribonucleic acid (RNA) or deoxyribonucleic acid (DNA) or
mimetics thereof. This term includes oligonucleotides
5   composed of naturally-occurring nucleobases, sugars and
covalent internucleoside (backbone) linkages as well as
oligonucleotides having non-naturally-occurring portions
which function similarly. Such modified or substituted
oligonucleotides are often preferred over native forms
10 because of desirable properties such as, for example,
enhanced cellular uptake, enhanced affinity for nucleic
acid target and increased stability in the presence of
nucleases.

While antisense oligonucleotides are a preferred form
15 of antisense compound, the present invention comprehends
other oligomeric antisense compounds, including but not
limited to oligonucleotide mimetics such as are described
below. The antisense compounds in accordance with this
invention preferably comprise from about 8 to about 30
20 nucleobases (i.e. from about 8 to about 30 linked
nucleosides). Particularly preferred antisense compounds
are antisense oligonucleotides, even more preferably those
comprising from about 12 to about 25 nucleobases. As is
known in the art, a nucleoside is a base-sugar combination.
25 The base portion of the nucleoside is normally a
heterocyclic base. The two most common classes of such
heterocyclic bases are the purines and the pyrimidines.
Nucleotides are nucleosides that further include a
phosphate group covalently linked to the sugar portion of
30 the nucleoside. For those nucleosides that include a
pentofuranosyl sugar, the phosphate group can be linked to
either the 2', 3' or 5' hydroxyl moiety of the sugar. In
forming oligonucleotides, the phosphate groups covalently
link adjacent nucleosides to one another to form a linear
35 polymeric compound. In turn the respective ends of this

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linear polymeric structure can be further joined to form a
circular structure, however, open linear structures are
generally preferred. Within the oligonucleotide structure,
the phosphate groups are commonly referred to as forming
5 the internucleoside backbone of the oligonucleotide. The
normal linkage or backbone of RNA and DNA is a 3' to 5'
phosphodiester linkage.

Specific examples of preferred antisense compounds
useful in this invention include oligonucleotides
10 containing modified backbones or non-natural
internucleoside linkages. As defined in this
specification, oligonucleotides having modified backbones
include those that retain a phosphorus atom in the backbone
and those that do not have a phosphorus atom in the
15 backbone. For the purposes of this specification, and as
sometimes referenced in the art, modified oligonucleotides
that do not have a phosphorus atom in their internucleoside
backbone can also be considered to be oligonucleosides.

Preferred modified oligonucleotide backbones include,
20 for example, phosphorothioates, chiral phosphorothioates,
phosphorodithioates, phosphotriesters, aminoalkyl-
phosphotriesters, methyl and other alkyl phosphonates
including 3'-alkylene phosphonates and chiral phosphonates,
phosphinates, phosphoramidates including 3'-amino
25 phosphoramidate and aminoalkylphosphoramidates,
thionophosphoramidates, thionoalkylphosphonates,
thionoalkylphosphotriesters, and boranophosphates having
normal 3'-5' linkages, 2'-5' linked analogs of these, and
those having inverted polarity wherein the adjacent pairs
30 of nucleoside units are linked 3'-5' to 5'-3' or 2'-5' to
5'-2'. Various salts, mixed salts and free acid forms are
also included.

Representative United States patents that teach the
preparation of the above phosphorus-containing linkages
35 include, but are not limited to, U.S.: 3,687,808;

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4,469,863; 4,476,301; 5,023,243; 5,177,196; 5,188,897;

5,264,423; 5,276,019; 5,278,302; 5,286,717; 5,321,131;

5,399,676; 5,405,939; 5,453,496; 5,455,233; 5,466,677;

5,476,925; 5,519,126; 5,536,821; 5,541,306; 5,550,111;

5   5,563,253; 5,571,799; 5,587,361; and 5,625,050, certain of
which are commonly owned with this application, and each of
which is herein incorporated by reference.

Preferred modified oligonucleotide backbones that do
not include a phosphorus atom therein have backbones that
10   are formed by short chain alkyl or cycloalkyl
internucleoside linkages, mixed heteroatom and alkyl or
cycloalkyl internucleoside linkages, or one or more short
chain heteroatomic or heterocyclic internucleoside
linkages. These include those having morpholino linkages
15   (formed in part from the sugar portion of a nucleoside);
siloxane backbones; sulfide, sulfoxide and sulfone
backbones; formacetyl and thioformacetyl backbones;
methylene formacetyl and thioformacetyl backbones; alkene
containing backbones; sulfamate backbones; methyleneimino
20   and methylenehydrazino backbones; sulfonate and sulfonamide
backbones; amide backbones; and others having mixed N, O, S
and CH_2 component parts.

Representative United States patents that teach the
preparation of the above oligonucleosides include, but are
25   not limited to, U.S.: 5,034,506; 5,166,315; 5,185,444;
5,214,134; 5,216,141; 5,235,033; 5,264,562; 5,264,564;
5,405,938; 5,434,257; 5,466,677; 5,470,967; 5,489,677;
5,541,307; 5,561,225; 5,596,086; 5,602,240; 5,610,289;
5,602,240; 5,608,046; 5,610,289; 5,618,704; 5,623,070;
30   5,663,312; 5,633,360; 5,677,437; and 5,677,439, certain of
which are commonly owned with this application, and each of
which is herein incorporated by reference.

In other preferred oligonucleotide mimetics, both the
sugar and the internucleoside linkage, i.e., the backbone,
35   of the nucleotide units are replaced with novel groups.

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The base units are maintained for hybridization with an
appropriate nucleic acid target compound. One such
oligomeric compound, an oligonucleotide mimetic that has
been shown to have excellent hybridization properties, is
5   referred to as a peptide nucleic acid (PNA). In PNA
compounds, the sugar-backbone of an oligonucleotide is
replaced with an amide containing backbone, in particular
an aminoethylglycine backbone. The nucleobases are
retained and are bound directly or indirectly to aza
10   nitrogen atoms of the amide portion of the backbone.
Representative United States patents that teach the
preparation of PNA compounds include, but are not limited
to, U.S.: 5,539,082; 5,714,331; and 5,719,262, each of
which is herein incorporated by reference. Further teaching
15   of PNA compounds can be found in Nielsen et al., *Science*,
1991, 254, 1497-1500.

Most preferred embodiments of the invention are
oligonucleotides with phosphorothioate backbones and
oligonucleosides with heteroatom backbones, and in
20   particular -CH_2-NH-O-CH_2-, -CH_2-N(CH_3)-O-CH_2- [known as a
methylene (methylimino) or MMI backbone], -CH_2-O-N(CH_3)-CH_2-,
-CH_2-N(CH_3)-N(CH_3)-CH_2- and -O-N(CH_3)-CH_2-CH_2- [wherein the
native phosphodiester backbone is represented as -O-P-O-CH_2-
] of the above referenced U.S. patent 5,489,677, and the
25   amide backbones of the above referenced U.S. patent
5,602,240. Also preferred are oligonucleotides having
morpholino backbone structures of the above-referenced U.S.
patent 5,034,506.

Modified oligonucleotides may also contain one or more
30   substituted sugar moieties. Preferred oligonucleotides
comprise one of the following at the 2' position: OH; F; O-
, S-, or N-alkyl; O-, S-, or N-alkenyl; O-, S- or N-
alkynyl; or O-alkyl-O-alkyl, wherein the alkyl, alkenyl and
alkynyl may be substituted or unsubstituted C_1 to C_{10} alkyl
35   or C_2 to C_{10} alkenyl and alkynyl. Particularly preferred are

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$O[(CH_2)_nO]_mCH_3$, $O(CH_2)_nOCH_3$, $O(CH_2)_nNH_2$, $O(CH_2)_nCH_3$, $O(CH_2)_nONH_2$, and $O(CH_2)_nON[(CH_2)_nCH_3)]_2$, where n and m are from 1 to about 10. Other preferred oligonucleotides comprise one of the following at the 2' position: C_1 to C_{10} lower alkyl,

5   substituted lower alkyl, alkaryl, aralkyl, O-alkaryl or O-aralkyl, SH, SCH_3, OCN, Cl, Br, CN, CF_3, OCF_3, $SOCH_3$, SO_2CH_3, ONO_2, NO_2, N_3, NH_2, heterocycloalkyl, heterocycloalkaryl, aminoalkylamino, polyalkylamino, substituted silyl, an RNA cleaving group, a reporter group, an intercalator, a group

10   for improving the pharmacokinetic properties of an oligonucleotide, or a group for improving the pharmacodynamic properties of an oligonucleotide, and other substituents having similar properties. A preferred modification includes 2'-methoxyethoxy ($2'-O-CH_2CH_2OCH_3$, also

15   known as 2'-O-(2-methoxyethyl) or 2'-MOE) (Martin et al., *Helv. Chim. Acta*, 1995, *78*, 486-504) i.e., an alkoxyalkoxy group. A further preferred modification includes 2'-dimethylaminooxyethoxy, i.e., a $O(CH_2)_2ON(CH_3)_2$ group, also known as 2'-DMAOE, as described in examples hereinbelow,

20   and 2'-dimethylaminoethoxyethoxy (also known in the art as 2'-O-dimethylaminoethoxyethyl or 2'-DMAEOE), i.e., $2'-O-CH_2-O-CH_2-N(CH_2)_2$, also described in examples hereinbelow.

       Other preferred modifications include 2'-methoxy (2'-$O-CH_3$), 2'-aminopropoxy ($2'-OCH_2CH_2CH_2NH_2$) and 2'-fluoro (2'-

25   F). Similar modifications may also be made at other positions on the oligonucleotide, particularly the 3' position of the sugar on the 3' terminal nucleotide or in 2'-5' linked oligonucleotides and the 5' position of 5' terminal nucleotide. Oligonucleotides may also have sugar

30   mimetics such as cyclobutyl moieties in place of the pentofuranosyl sugar. Representative United States patents that teach the preparation of such modified sugar structures include, but are not limited to, U.S.: 4,981,957; 5,118,800; 5,319,080; 5,359,044; 5,393,878;

35   5,446,137; 5,466,786; 5,514,785; 5,519,134; 5,567,811;

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5,576,427; 5,591,722; 5,597,909; 5,610,300; 5,627,053;
5,639,873; 5,646,265; 5,658,873; 5,670,633; and 5,700,920,
certain of which are commonly owned with the instant
application, and each of which is herein incorporated by
5   reference in its entirety.

Oligonucleotides may also include nucleobase (often
referred to in the art simply as "base") modifications or
substitutions. As used herein, "unmodified" or "natural"
nucleobases include the purine bases adenine (A) and
10   guanine (G), and the pyrimidine bases thymine (T), cytosine
(C) and uracil (U). Modified nucleobases include other
synthetic and natural nucleobases such as 5-methylcytosine
(5-me-C), 5-hydroxymethyl cytosine, xanthine, hypoxanthine,
2-aminoadenine, 6-methyl and other alkyl derivatives of
15   adenine and guanine, 2-propyl and other alkyl derivatives
of adenine and guanine, 2-thiouracil, 2-thiothymine and 2-
thiocytosine, 5-halouracil and cytosine, 5-propynyl uracil
and cytosine, 6-azo uracil, cytosine and thymine, 5-uracil
(pseudouracil), 4-thiouracil, 8-halo, 8-amino, 8-thiol, 8-
20   thioalkyl, 8-hydroxyl and other 8-substituted adenines and
guanines, 5-halo particularly 5-bromo, 5-trifluoromethyl
and other 5-substituted uracils and cytosines, 7-
methylguanine and 7-methyladenine, 8-azaguanine and 8-
azaadenine, 7-deazaguanine and 7-deazaadenine and 3-
25   deazaguanine and 3-deazaadenine. Further nucleobases
include those disclosed in United States Patent No.
3,687,808, those disclosed in *The Concise Encyclopedia Of
Polymer Science And Engineering*, pages 858-859, Kroschwitz,
J.I., ed. John Wiley & Sons, 1990, those disclosed by
30   Englisch et al., *Angewandte Chemie*, International Edition,
1991, *30*, 613, and those disclosed by Sanghvi, Y.S.,
Chapter 15, *Antisense Research and Applications*, pages 289-
302, Crooke, S.T. and Lebleu, B. , ed., CRC Press, 1993.
Certain of these nucleobases are particularly useful for

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increasing the binding affinity of the oligomeric compounds of the invention. These include 5-substituted pyrimidines, 6-azapyrimidines and N-2, N-6 and O-6 substituted purines, including 2-aminopropyladenine, 5-propynyluracil and 5-

5   propynylcytosine. 5-methylcytosine substitutions have been shown to increase nucleic acid duplex stability by 0.6-1.2°C (Sanghvi, Y.S., Crooke, S.T. and Lebleu, B., eds., *Antisense Research and Applications*, CRC Press, Boca Raton, 1993, pp. 276-278) and are presently preferred base

10 substitutions, even more particularly when combined with 2'-O-methoxyethyl sugar modifications.

Representative United States patents that teach the preparation of certain of the above noted modified nucleobases as well as other modified nucleobases include,

15 but are not limited to, the above noted U.S. 3,687,808, as well as U.S.: 4,845,205; 5,130,302; 5,134,066; 5,175,273; 5,367,066; 5,432,272; 5,457,187; 5,459,255; 5,484,908; 5,502,177; 5,525,711; 5,552,540; 5,587,469; 5,594,121, 5,596,091; 5,614,617; and 5,681,941, certain of which are

20 commonly owned with the instant application, and each of which is herein incorporated by reference, and United States patent 5,750,692, which is commonly owned with the instant application and also herein incorporated by reference.

25 Another modification of the oligonucleotides of the invention involves chemically linking to the oligonucleotide one or more moieties or conjugates which enhance the activity, cellular distribution or cellular uptake of the oligonucleotide. Such moieties include but

30 are not limited to lipid moieties such as a cholesterol moiety (Letsinger et al., *Proc. Natl. Acad. Sci. USA*, 1989, *86*, 6553-6556), cholic acid (Manoharan et al., *Bioorg. Med. Chem. Let.*, 1994, *4*, 1053-1060), a thioether, e.g., hexyl-S-tritylthiol (Manoharan et al., *Ann. N.Y. Acad. Sci.*,

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1992, *660*, 306-309; Manoharan et al., *Bioorg. Med. Chem. Let.*, **1993**, *3*, 2765-2770), a thiocholesterol (Oberhauser et al., *Nucl. Acids Res.*, **1992**, *20*, 533-538), an aliphatic chain, e.g., dodecandiol or undecyl residues (Saison-Behmoaras et al., *EMBO J.*, **1991**, *10*, 1111-1118; Kabanov et al., *FEBS Lett.*, **1990**, *259*, 327-330; Svinarchuk et al., *Biochimie*, **1993**, *75*, 49-54), a phospholipid, e.g., di-hexadecyl-rac-glycerol or triethylammonium 1,2-di-O-hexadecyl-rac-glycero-3-H-phosphonate (Manoharan et al., *Tetrahedron Lett.*, **1995**, *36*, 3651-3654; Shea et al., *Nucl. Acids Res.*, **1990**, *18*, 3777-3783), a polyamine or a polyethylene glycol chain (Manoharan et al., *Nucleosides & Nucleotides*, **1995**, *14*, 969-973), or adamantane acetic acid (Manoharan et al., *Tetrahedron Lett.*, **1995**, *36*, 3651-3654), a palmityl moiety (Mishra et al., *Biochim. Biophys. Acta*, **1995**, *1264*, 229-237), or an octadecylamine or hexylamino-carbonyl-oxycholesterol moiety (Crooke et al., *J. Pharmacol. Exp. Ther.*, **1996**, *277*, 923-937.

Representative United States patents that teach the preparation of such oligonucleotide conjugates include, but are not limited to, U.S.: 4,828,979; 4,948,882; 5,218,105; 5,525,465; 5,541,313; 5,545,730; 5,552,538; 5,578,717, 5,580,731; 5,580,731; 5,591,584; 5,109,124; 5,118,802; 5,138,045; 5,414,077; 5,486,603; 5,512,439; 5,578,718; 5,608,046; 4,587,044; 4,605,735; 4,667,025; 4,762,779; 4,789,737; 4,824,941; 4,835,263; 4,876,335; 4,904,582; 4,958,013; 5,082,830; 5,112,963; 5,214,136; 5,082,830; 5,112,963; 5,214,136; 5,245,022; 5,254,469; 5,258,506; 5,262,536; 5,272,250; 5,292,873; 5,317,098; 5,371,241, 5,391,723; 5,416,203, 5,451,463; 5,510,475; 5,512,667; 5,514,785; 5,565,552; 5,567,810; 5,574,142; 5,585,481; 5,587,371; 5,595,726; 5,597,696; 5,599,923; 5,599,928 and 5,688,941, certain of which are commonly owned with the

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instant application, and each of which is herein
incorporated by reference.

It is not necessary for all positions in a given
compound to be uniformly modified, and in fact more than
5   one of the aforementioned modifications may be incorporated
in a single compound or even at a single nucleoside within
an oligonucleotide. The present invention also includes
antisense compounds which are chimeric compounds.
"Chimeric" antisense compounds or "chimeras," in the
10   context of this invention, are antisense compounds,
particularly oligonucleotides, which contain two or more
chemically distinct regions, each made up of at least one
monomer unit, i.e., a nucleotide in the case of an
oligonucleotide compound. These oligonucleotides typically
15   contain at least one region wherein the oligonucleotide is
modified so as to confer upon the oligonucleotide increased
resistance to nuclease degradation, increased cellular
uptake, and/or increased binding affinity for the target
nucleic acid. An additional region of the oligonucleotide
20   may serve as a substrate for enzymes capable of cleaving
RNA:DNA or RNA:RNA hybrids. By way of example, RNase H is
a cellular endonuclease which cleaves the RNA strand of an
RNA:DNA duplex. Activation of RNase H, therefore, results
in cleavage of the RNA target, thereby greatly enhancing
25   the efficiency of oligonucleotide inhibition of gene
expression. Consequently, comparable results can often be
obtained with shorter oligonucleotides when chimeric
oligonucleotides are used, compared to phosphorothioate
deoxyoligonucleotides hybridizing to the same target
30   region. Cleavage of the RNA target can be routinely
detected by gel electrophoresis and, if necessary,
associated nucleic acid hybridization techniques known in
the art.

Chimeric antisense compounds of the invention may be
35   formed as composite structures of two or more

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oligonucleotides, modified oligonucleotides, oligonucleosides and/or oligonucleotide mimetics as described above. Such compounds have also been referred to in the art as hybrids or gapmers. Representative United

5   States patents that teach the preparation of such hybrid structures include, but are not limited to, U.S.: 5,013,830; 5,149,797; 5,220,007; 5,256,775; 5,366,878; 5,403,711; 5,491,133; 5,565,350; 5,623,065; 5,652,355; 5,652,356; and 5,700,922, certain of which are commonly

10   owned with the instant application, and each of which is herein incorporated by reference in its entirety.

The antisense compounds used in accordance with this invention may be conveniently and routinely made through the well-known technique of solid phase synthesis.

15   Equipment for such synthesis is sold by several vendors including, for example, Applied Biosystems (Foster City, CA). Any other means for such synthesis known in the art may additionally or alternatively be employed. It is well known to use similar techniques to prepare oligonucleotides

20   such as the phosphorothioates and alkylated derivatives.

The antisense compounds of the invention are synthesized in vitro and do not include antisense compositions of biological origin, or genetic vector constructs designed to direct the in vivo synthesis of

25   antisense molecules.

The compounds of the invention may also be admixed, encapsulated, conjugated or otherwise associated with other molecules, molecule structures or mixtures of compounds, as for example, liposomes, receptor targeted molecules, oral,

30   rectal, topical or other formulations, for assisting in uptake, distribution and/or absorption. Representative United States patents that teach the preparation of such uptake, distribution and/or absorption assisting formulations include, but are not limited to, U.S.:

35   5,108,921; 5,354,844; 5,416,016; 5,459,127; 5,521,291;

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5,543,158; 5,547,932; 5,583,020; 5,591,721; 4,426,330;

4,534,899; 5,013,556; 5,108,921; 5,213,804; 5,227,170;

5,264,221; 5,356,633; 5,395,619; 5,416,016; 5,417,978;

5,462,854; 5,469,854; 5,512,295; 5,527,528; 5,534,259;

5   5,543,152; 5,556,948; 5,580,575; and 5,595,756, each of

which is herein incorporated by reference.

The antisense compounds of the invention encompass any

pharmaceutically acceptable salts, esters, or salts of such

esters, or any other compound which, upon administration to

10   an animal including a human, is capable of providing

(directly or indirectly) the biologically active metabolite

or residue thereof. Accordingly, for example, the

disclosure is also drawn to prodrugs and pharmaceutically

acceptable salts of the compounds of the invention,

15   pharmaceutically acceptable salts of such prodrugs, and

other bioequivalents.

The term "prodrug" indicates a therapeutic agent that

is prepared in an inactive form that is converted to an

active form (i.e., drug) within the body or cells thereof

20   by the action of endogenous enzymes or other chemicals

and/or conditions. In particular, prodrug versions of the

oligonucleotides of the invention are prepared as SATE

[(S-acetyl-2-thioethyl) phosphate] derivatives according to

the methods disclosed in WO 93/24510 to Gosselin et al.,

25   published December 9, 1993 or in WO 94/26764 to Imbach et

al.

The term "pharmaceutically acceptable salts" refers to

physiologically and pharmaceutically acceptable salts of

the compounds of the invention: i.e., salts that retain the

30   desired biological activity of the parent compound and do

not impart undesired toxicological effects thereto.

Pharmaceutically acceptable base addition salts are

formed with metals or amines, such as alkali and alkaline

earth metals or organic amines. Examples of metals used as

35   cations are sodium, potassium, magnesium, calcium, and the

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like. Examples of suitable amines are
N,N'-dibenzylethylenediamine, chloroprocaine, choline,
diethanolamine, dicyclohexylamine, ethylenediamine,
N-methylglucamine, and procaine (see, for example, Berge et

5   al., "Pharmaceutical Salts," *J. of Pharma Sci.*, 1977, *66*,
1-19). The base addition salts of said acidic compounds
are prepared by contacting the free acid form with a
sufficient amount of the desired base to produce the salt
in the conventional manner. The free acid form may be

10   regenerated by contacting the salt form with an acid and
isolating the free acid in the conventional manner. The
free acid forms differ from their respective salt forms
somewhat in certain physical properties such as solubility
in polar solvents, but otherwise the salts are equivalent

15   to their respective free acid for purposes of the present
invention. As used herein, a "pharmaceutical addition
salt" includes a pharmaceutically acceptable salt of an
acid form of one of the components of the compositions of
the invention. These include organic or inorganic acid

20   salts of the amines. Preferred acid salts are the
hydrochlorides, acetates, salicylates, nitrates and
phosphates. Other suitable pharmaceutically acceptable
salts are well known to those skilled in the art and
include basic salts of a variety of inorganic and organic

25   acids, such as, for example, with inorganic acids, such as
for example hydrochloric acid, hydrobromic acid, sulfuric
acid or phosphoric acid; with organic carboxylic, sulfonic,
sulfo or phospho acids or N-substituted sulfamic acids, for
example acetic acid, propionic acid, glycolic acid,

30   succinic acid, maleic acid, hydroxymaleic acid,
methylmaleic acid, fumaric acid, malic acid, tartaric acid,
lactic acid, oxalic acid, gluconic acid, glucaric acid,
glucuronic acid, citric acid, benzoic acid, cinnamic acid,
mandelic acid, salicylic acid, 4-aminosalicylic acid,

35   2-phenoxybenzoic acid, 2-acetoxybenzoic acid, embonic acid,

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nicotinic acid or isonicotinic acid; and with amino acids, such as the 20 alpha-amino acids involved in the synthesis of proteins in nature, for example glutamic acid or aspartic acid, and also with phenylacetic acid,
5   methanesulfonic acid, ethanesulfonic acid, 2-hydroxyethanesulfonic acid, ethane-1,2-disulfonic acid, benzenesulfonic acid, 4-methylbenzenesulfoic acid, naphthalene-2-sulfonic acid, naphthalene-1,5-disulfonic acid, 2- or 3-phosphoglycerate, glucose-6-phosphate,
10   N-cyclohexylsulfamic acid (with the formation of cyclamates), or with other acid organic compounds, such as ascorbic acid. Pharmaceutically acceptable salts of compounds may also be prepared with a pharmaceutically acceptable cation. Suitable pharmaceutically acceptable
15   cations are well known to those skilled in the art and include alkaline, alkaline earth, ammonium and quaternary ammonium cations. Carbonates or hydrogen carbonates are also possible.

     For oligonucleotides, preferred examples of
20   pharmaceutically acceptable salts include but are not limited to (a) salts formed with cations such as sodium, potassium, ammonium, magnesium, calcium, polyamines such as spermine and spermidine, etc.; (b) acid addition salts formed with inorganic acids, for example hydrochloric acid,
25   hydrobromic acid, sulfuric acid, phosphoric acid, nitric acid and the like; (c) salts formed with organic acids such as, for example, acetic acid, oxalic acid, tartaric acid, succinic acid, maleic acid, fumaric acid, gluconic acid, citric acid, malic acid, ascorbic acid, benzoic acid,
30   tannic acid, palmitic acid, alginic acid, polyglutamic acid, naphthalenesulfonic acid, methanesulfonic acid, p-toluenesulfonic acid, naphthalenedisulfonic acid, polygalacturonic acid, and the like; and (d) salts formed from elemental anions such as chlorine, bromine, and
35   iodine.

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The antisense compounds of the present invention can
be utilized for diagnostics, therapeutics, prophylaxis and
as research reagents and kits. For therapeutics, an
animal, preferably a human, suspected of having a disease

5   or disorder which can be treated by modulating the
expression of G-alpha-i3 is treated by administering
antisense compounds in accordance with this invention. The
compounds of the invention can be utilized in
pharmaceutical compositions by adding an effective amount

10   of an antisense compound to a suitable pharmaceutically
acceptable diluent or carrier. Use of the antisense
compounds and methods of the invention may also be useful
prophylactically, e.g., to prevent or delay infection,
inflammation or tumor formation, for example.

15   The antisense compounds of the invention are useful
for research and diagnostics, because these compounds
hybridize to nucleic acids encoding G-alpha-i3, enabling
sandwich and other assays to easily be constructed to
exploit this fact. Hybridization of the antisense

20   oligonucleotides of the invention with a nucleic acid
encoding G-alpha-i3 can be detected by means known in the
art. Such means may include conjugation of an enzyme to
the oligonucleotide, radiolabelling of the oligonucleotide
or any other suitable detection means. Kits using such

25   detection means for detecting the level of G-alpha-i3 in a
sample may also be prepared.

The present invention also includes pharmaceutical
compositions and formulations which include the antisense
compounds of the invention. The pharmaceutical

30   compositions of the present invention may be administered
in a number of ways depending upon whether local or
systemic treatment is desired and upon the area to be
treated. Administration may be topical (including
ophthalmic and to mucous membranes including vaginal and

35   rectal delivery), pulmonary, e.g., by inhalation or

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insufflation of powders or aerosols, including by
nebulizer; intratracheal, intranasal, epidermal and
transdermal), oral or parenteral. Parenteral
administration includes intravenous, intraarterial,
5   subcutaneous, intraperitoneal or intramuscular injection or
infusion; or intracranial, e.g., intrathecal or
intraventricular, administration. Oligonucleotides with at
least one 2'-O-methoxyethyl modification are believed to be
particularly useful for oral administration.
10   Pharmaceutical compositions and formulations for
topical administration may include transdermal patches,
ointments, lotions, creams, gels, drops, suppositories,
sprays, liquids and powders. Conventional pharmaceutical
carriers, aqueous, powder or oily bases, thickeners and the
15 like may be necessary or desirable. Coated condoms, gloves
and the like may also be useful.
        Compositions and formulations for oral administration
include powders or granules, suspensions or solutions in
water or non-aqueous media, capsules, sachets or tablets.
20 Thickeners, flavoring agents, diluents, emulsifiers,
dispersing aids or binders may be desirable.
        Compositions and formulations for parenteral,
intrathecal or intraventricular administration may include
sterile aqueous solutions which may also contain buffers,
25 diluents and other suitable additives such as, but not
limited to, penetration enhancers, carrier compounds and
other pharmaceutically acceptable carriers or excipients.
        Pharmaceutical compositions of the present invention
include, but are not limited to, solutions, emulsions, and
30 liposome-containing formulations. These compositions may
be generated from a variety of components that include, but
are not limited to, preformed liquids, self-emulsifying
solids and self-emulsifying semisolids.
        The pharmaceutical formulations of the present
35 invention, which may conveniently be presented in unit

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dosage form, may be prepared according to conventional
techniques well known in the pharmaceutical industry. Such
techniques include the step of bringing into association
the active ingredients with the pharmaceutical carrier(s)
5   or excipient(s). In general the formulations are prepared
by uniformly and intimately bringing into association the
active ingredients with liquid carriers or finely divided
solid carriers or both, and then, if necessary, shaping the
product.

10   The compositions of the present invention may be
formulated into any of many possible dosage forms such as,
but not limited to, tablets, capsules, liquid syrups, soft
gels, suppositories, and enemas. The compositions of the
present invention may also be formulated as suspensions in
15 aqueous, non-aqueous or mixed media. Aqueous suspensions
may further contain substances which increase the viscosity
of the suspension including, for example, sodium
carboxymethylcellulose, sorbitol and/or dextran. The
suspension may also contain stabilizers.

20   In one embodiment of the present invention the
pharmaceutical compositions may be formulated and used as
foams. Pharmaceutical foams include formulations such as,
but not limited to, emulsions, microemulsions, creams,
jellies and liposomes. While basically similar in nature
25 these formulations vary in the components and the
consistency of the final product. The preparation of such
compositions and formulations is generally known to those
skilled in the pharmaceutical and formulation arts and may
be applied to the formulation of the compositions of the
30 present invention.

Emulsions
     The compositions of the present invention may be
prepared and formulated as emulsions. Emulsions are
35 typically heterogenous systems of one liquid dispersed in

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another in the form of droplets usually exceeding 0.1 μm in
diameter. (Idson, in *Pharmaceutical Dosage Forms*,
Lieberman, Rieger and Banker (Eds.), **1988**, Marcel Dekker,
Inc., New York, N.Y., volume 1, p. 199; Rosoff, in
5   *Pharmaceutical Dosage Forms*, Lieberman, Rieger and Banker
(Eds.), **1988**, Marcel Dekker, Inc., New York, N.Y., Volume
1, p. 245; Block in *Pharmaceutical Dosage Forms*, Lieberman,
Rieger and Banker (Eds.), **1988**, Marcel Dekker, Inc., New
York, N.Y., volume 2, p. 335; Higuchi *et al.*, in
10   *Remington's Pharmaceutical Sciences*, Mack Publishing Co.,
Easton, PA, **1985**, p. 301). Emulsions are often biphasic
systems comprising of two immiscible liquid phases
intimately mixed and dispersed with each other. In
general, emulsions may be either water-in-oil (w/o) or of
15   the oil-in-water (o/w) variety. When an aqueous phase is
finely divided into and dispersed as minute droplets into a
bulk oily phase the resulting composition is called a
water-in-oil (w/o) emulsion. Alternatively, when an oily
phase is finely divided into and dispersed as minute
20   droplets into a bulk aqueous phase the resulting
composition is called an oil-in-water (o/w) emulsion.
Emulsions may contain additional components in addition to
the dispersed phases and the active drug which may be
present as a solution in either the aqueous phase, oily
25   phase or itself as a separate phase. Pharmaceutical
excipients such as emulsifiers, stabilizers, dyes, and
anti-oxidants may also be present in emulsions as needed.
Pharmaceutical emulsions may also be multiple emulsions
that are comprised of more than two phases such as, for
30   example, in the case of oil-in-water-in-oil (o/w/o) and
water-in-oil-in-water (w/o/w) emulsions. Such complex
formulations often provide certain advantages that simple
binary emulsions do not. Multiple emulsions in which
individual oil droplets of an o/w emulsion enclose small

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water droplets constitute a w/o/w emulsion. Likewise a
system of oil droplets enclosed in globules of water
stabilized in an oily continuous provides an o/w/o
emulsion.

5   Emulsions are characterized by little or no
thermodynamic stability. Often, the dispersed or
discontinuous phase of the emulsion is well dispersed into
the external or continuous phase and maintained in this
form through the means of emulsifiers or the viscosity of
10   the formulation. Either of the phases of the emulsion may
be a semisolid or a solid, as is the case of emulsion-style
ointment bases and creams. Other means of stabilizing
emulsions entail the use of emulsifiers that may be
incorporated into either phase of the emulsion.
15   Emulsifiers may broadly be classified into four categories:
synthetic surfactants, naturally occurring emulsifiers,
absorption bases, and finely dispersed solids (Idson, in
Pharmaceutical Dosage Forms, Lieberman, Rieger and Banker
(Eds.), **1988**, Marcel Dekker, Inc., New York, N.Y., volume
20   1, p. 199).

        Synthetic surfactants, also known as surface active
agents, have found wide applicability in the formulation of
emulsions and have been reviewed in the literature (Rieger,
in *Pharmaceutical Dosage Forms*, Lieberman, Rieger and
25   Banker (Eds.), **1988**, Marcel Dekker, Inc., New York, N.Y.,
volume 1, p. 285; Idson, in *Pharmaceutical Dosage Forms*,
Lieberman, Rieger and Banker (Eds.), Marcel Dekker, Inc.,
New York, N.Y., **1988**, volume 1, p. 199). Surfactants are
typically amphiphilic and comprise a hydrophilic and a
30   hydrophobic portion. The ratio of the hydrophilic to the
hydrophobic nature of the surfactant has been termed the
hydrophile/lipophile balance (HLB) and is a valuable tool
in categorizing and selecting surfactants in the
preparation of formulations. Surfactants may be classified
35   into different classes based on the nature of the

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hydrophilic group: nonionic, anionic, cationic and
amphoteric (Rieger, in *Pharmaceutical Dosage Forms*,
Lieberman, Rieger and Banker (Eds.), **1988**, Marcel Dekker,
Inc., New York, N.Y., volume 1, p. 285).

5   Naturally occurring emulsifiers used in emulsion
formulations include lanolin, beeswax, phosphatides,
lecithin and acacia. Absorption bases possess hydrophilic
properties such that they can soak up water to form w/o
emulsions yet retain their semisolid consistencies, such as
10   anhydrous lanolin and hydrophilic petrolatum. Finely
divided solids have also been used as good emulsifiers
especially in combination with surfactants and in viscous
preparations. These include polar inorganic solids, such
as heavy metal hydroxides, nonswelling clays such as
15   bentonite, attapulgite, hectorite, kaolin, montmorillonite,
colloidal aluminum silicate and colloidal magnesium
aluminum silicate, pigments and nonpolar solids such as
carbon or glyceryl tristearate.

           A large variety of non-emulsifying materials are also
20   included in emulsion formulations and contribute to the
properties of emulsions. These include fats, oils, waxes,
fatty acids, fatty alcohols, fatty esters, humectants,
hydrophilic colloids, preservatives and antioxidants
(Block, in *Pharmaceutical Dosage Forms*, Lieberman, Rieger
25   and Banker (Eds.), **1988**, Marcel Dekker, Inc., New York,
N.Y., volume 1, p. 335; Idson, in *Pharmaceutical Dosage
Forms*, Lieberman, Rieger and Banker (Eds.), **1988**, Marcel
Dekker, Inc., New York, N.Y., volume 1, p. 199).

           Hydrophilic colloids or hydrocolloids include
30   naturally occurring gums and synthetic polymers such as
polysaccharides (for example, acacia, agar, alginic acid,
carrageenan, guar gum, karaya gum, and tragacanth),
cellulose derivatives (for example, carboxymethylcellulose
and carboxypropylcellulose), and synthetic polymers (for
35   example, carbomers, cellulose ethers, and carboxyvinyl

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polymers). These disperse or swell in water to form
colloidal solutions that stabilize emulsions by forming
strong interfacial films around the dispersed-phase
droplets and by increasing the viscosity of the external
5   phase.

Since emulsions often contain a number of ingredients
such as carbohydrates, proteins, sterols and phosphatides
that may readily support the growth of microbes, these
formulations often incorporate preservatives. Commonly
10   used preservatives included in emulsion formulations
include methyl paraben, propyl paraben, quaternary ammonium
salts, benzalkonium chloride, esters of p-hydroxybenzoic
acid, and boric acid. Antioxidants are also commonly added
to emulsion formulations to prevent deterioration of the
15   formulation. Antioxidants used may be free radical
scavengers such as tocopherols, alkyl gallates, butylated
hydroxyanisole, butylated hydroxytoluene, or reducing
agents such as ascorbic acid and sodium metabisulfite, and
antioxidant synergists such as citric acid, tartaric acid,
20   and lecithin.

The application of emulsion formulations via
dermatological, oral and parenteral routes and methods for
their manufacture have been reviewed in the literature
(Idson, in *Pharmaceutical Dosage Forms*, Lieberman, Rieger
25   and Banker (Eds.), **1988**, Marcel Dekker, Inc., New York,
N.Y., volume 1, p. 199). Emulsion formulations for oral
delivery have been very widely used because of reasons of
ease of formulation, efficacy from an absorption and
bioavailability standpoint. (Rosoff, in *Pharmaceutical*
30   *Dosage Forms*, Lieberman, Rieger and Banker (Eds.), **1988**,
Marcel Dekker, Inc., New York, N.Y., volume 1, p. 245;
Idson, in *Pharmaceutical Dosage Forms*, Lieberman, Rieger
and Banker (Eds.), **1988**, Marcel Dekker, Inc., New York,
N.Y., volume 1, p. 199). Mineral-oil base laxatives, oil-
35   soluble vitamins and high fat nutritive preparations are

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among the materials that have commonly been administered orally as o/w emulsions.

    In one embodiment of the present invention, the compositions of oligonucleotides and nucleic acids are
5   formulated as microemulsions. A microemulsion may be defined as a system of water, oil and amphiphile which is a single optically isotropic and thermodynamically stable liquid solution (Rosoff, in *Pharmaceutical Dosage Forms*, Lieberman, Rieger and Banker (Eds.), **1988**, Marcel Dekker,
10 Inc., New York, N.Y., volume 1, p. 245). Typically microemulsions are systems that are prepared by first dispersing an oil in an aqueous surfactant solution and then adding a sufficient amount of a fourth component, generally an intermediate chain-length alcohol to form a
15 transparent system. Therefore, microemulsions have also been described as thermodynamically stable, isotropically clear dispersions of two immiscible liquids that are stabilized by interfacial films of surface-active molecules (Leung and Shah, *in: Controlled Release of Drugs: Polymers*
20 *and Aggregate Systems*, Rosoff, M., Ed., **1989**, VCH Publishers, New York, pages 185-215). Microemulsions commonly are prepared via a combination of three to five components that include oil, water, surfactant, cosurfactant and electrolyte. Whether the microemulsion is
25 of the water-in-oil (w/o) or an oil-in-water (o/w) type is dependent on the properties of the oil and surfactant used and on the structure and geometric packing of the polar heads and hydrocarbon tails of the surfactant molecules (Schott, in *Remington's Pharmaceutical Sciences*, Mack
30 Publishing Co., Easton, PA, **1985**, p. 271).

    The phenomenological approach utilizing phase diagrams has been extensively studied and has yielded a comprehensive knowledge, to one skilled in the art, of how to formulate microemulsions (Rosoff, in *Pharmaceutical*

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Dosage Forms, Lieberman, Rieger and Banker (Eds.), **1988**,
Marcel Dekker, Inc., New York, N.Y., volume 1, p. 245;
Block, in *Pharmaceutical Dosage Forms*, Lieberman, Rieger
and Banker (Eds.), **1988**, Marcel Dekker, Inc., New York,

5   N.Y., volume 1, p. 335). Compared to conventional
emulsions, microemulsions offer the advantage of
solubilizing water-insoluble drugs in a formulation of
thermodynamically stable droplets that are formed
spontaneously.

10   Surfactants used in the preparation of microemulsions
include, but are not limited to, ionic surfactants, non-
ionic surfactants, Brij 96, polyoxyethylene oleyl ethers,
polyglycerol fatty acid esters, tetraglycerol monolaurate
(ML310), tetraglycerol monooleate (MO310), hexaglycerol

15   monooleate (PO310), hexaglycerol pentaoleate (PO500),
decaglycerol monocaprate (MCA750), decaglycerol monooleate
(MO750), decaglycerol sequioleate (SO750), decaglycerol
decaoleate (DAO750), alone or in combination with
cosurfactants. The cosurfactant, usually a short-chain

20   alcohol such as ethanol, 1-propanol, and 1-butanol, serves
to increase the interfacial fluidity by penetrating into
the surfactant film and consequently creating a disordered
film because of the void space generated among surfactant
molecules. Microemulsions may, however, be prepared

25   without the use of cosurfactants and alcohol-free self-
emulsifying microemulsion systems are known in the art.
The aqueous phase may typically be, but is not limited to,
water, an aqueous solution of the drug, glycerol, PEG300,
PEG400, polyglycerols, propylene glycols, and derivatives

30   of ethylene glycol. The oil phase may include, but is not
limited to, materials such as Captex 300, Captex 355,
Capmul MCM, fatty acid esters, medium chain (C8-C12) mono,
di, and tri-glycerides, polyoxyethylated glyceryl fatty
acid esters, fatty alcohols, polyglycolized glycerides,

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saturated polyglycolized C8-C10 glycerides, vegetable oils
and silicone oil.

    Microemulsions are particularly of interest from the
standpoint of drug solubilization and the enhanced
5   absorption of drugs. Lipid based microemulsions (both o/w
and w/o) have been proposed to enhance the oral
bioavailability of drugs, including peptides
(Constantinides et al., *Pharmaceutical Research*, **1994**, *11*,
1385-1390; Ritschel, *Meth. Find. Exp. Clin. Pharmacol.*,
10 **1993**, *13*, 205). Microemulsions afford advantages of
improved drug solubilization, protection of drug from
enzymatic hydrolysis, possible enhancement of drug
absorption due to surfactant-induced alterations in
membrane fluidity and permeability, ease of preparation,
15 ease of oral administration over solid dosage forms,
improved clinical potency, and decreased toxicity
(Constantinides et al., *Pharmaceutical Research*, **1994**, *11*,
1385; Ho *et al.*, *J. Pharm. Sci.*, **1996**, *85*, 138-143). Often
microemulsions may form spontaneously when their components
20 are brought together at ambient temperature. This may be
particularly advantageous when formulating thermolabile
drugs, peptides or oligonucleotides. Microemulsions have
also been effective in the transdermal delivery of active
components in both cosmetic and pharmaceutical
25 applications. It is expected that the microemulsion
compositions and formulations of the present invention will
facilitate the increased systemic absorption of
oligonucleotides and nucleic acids from the
gastrointestinal tract, as well as improve the local
30 cellular uptake of oligonucleotides and nucleic acids
within the gastrointestinal tract, vagina, buccal cavity
and other areas of administration.

    Microemulsions of the present invention may also
contain additional components and additives such as

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sorbitan monostearate (Grill 3), Labrasol, and penetration
enhancers to improve the properties of the formulation and
to enhance the absorption of the oligonucleotides and
nucleic acids of the present invention. Penetration
5   enhancers used in the microemulsions of the present
invention may be classified as belonging to one of five
broad categories - surfactants, fatty acids, bile salts,
chelating agents, and non-chelating non-surfactants (Lee *et
al.*, *Critical Reviews in Therapeutic Drug Carrier Systems*,
10   **1991**, p. 92). Each of these classes has been discussed
above.

Liposomes

        There are many organized surfactant structures besides
15   microemulsions that have been studied and used for the
formulation of drugs. These include monolayers, micelles,
bilayers and vesicles. Vesicles, such as liposomes, have
attracted great interest because of their specificity and
the duration of action they offer from the standpoint of
20   drug delivery. As used in the present invention, the term
"liposome" means a vesicle composed of amphiphilic lipids
arranged in a spherical bilayer or bilayers.

        Liposomes are unilamellar or multilamellar vesicles
which have a membrane formed from a lipophilic material and
25   an aqueous interior. The aqueous portion contains the
composition to be delivered. Cationic liposomes possess
the advantage of being able to fuse to the cell wall. Non-
cationic liposomes, although not able to fuse as
efficiently with the cell wall, are taken up by macrophages
30   *in vivo.*

        In order to cross intact mammalian skin, lipid
vesicles must pass through a series of fine pores, each
with a diameter less than 50 nm, under the influence of a
suitable transdermal gradient. Therefore, it is desirable

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to use a liposome which is highly deformable and able to pass through such fine pores.

Further advantages of liposomes include; liposomes obtained from natural phospholipids are biocompatible and

5   biodegradable; liposomes can incorporate a wide range of water and lipid soluble drugs; liposomes can protect encapsulated drugs in their internal compartments from metabolism and degradation (Rosoff, in *Pharmaceutical Dosage Forms*, Lieberman, Rieger and Banker (Eds.), **1988**,

10 Marcel Dekker, Inc., New York, N.Y., volume 1, p. 245). Important considerations in the preparation of liposome formulations are the lipid surface charge, vesicle size and the aqueous volume of the liposomes.

Liposomes are useful for the transfer and delivery of

15 active ingredients to the site of action. Because the liposomal membrane is structurally similar to biological membranes, when liposomes are applied to a tissue, the liposomes start to merge with the cellular membranes. As the merging of the liposome and cell progresses, the

20 liposomal contents are emptied into the cell where the active agent may act.

Liposomal formulations have been the focus of extensive investigation as the mode of delivery for many drugs. There is growing evidence that for topical

25 administration, liposomes present several advantages over other formulations. Such advantages include reduced side-effects related to high systemic absorption of the administered drug, increased accumulation of the administered drug at the desired target, and the ability to

30 administer a wide variety of drugs, both hydrophilic and hydrophobic, into the skin.

Several reports have detailed the ability of liposomes to deliver agents including high-molecular weight DNA into the skin. Compounds including analgesics, antibodies,

35 hormones and high-molecular weight DNAs have been

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administered to the skin. The majority of applications
resulted in the targeting of the upper epidermis.

    Liposomes fall into two broad classes. Cationic
liposomes are positively charged liposomes which interact
5   with the negatively charged DNA molecules to form a stable
complex. The positively charged DNA/liposome complex binds
to the negatively charged cell surface and is internalized
in an endosome. Due to the acidic pH within the endosome,
the liposomes are ruptured, releasing their contents into
10 the cell cytoplasm (Wang et al., Biochem. Biophys. Res.
Commun., 1987, 147, 980-985).

    Liposomes which are pH-sensitive or
negatively-charged, entrap DNA rather than complex with it.
Since both the DNA and the lipid are similarly charged,
15 repulsion rather than complex formation occurs.
Nevertheless, some DNA is entrapped within the aqueous
interior of these liposomes. pH-sensitive liposomes have
been used to deliver DNA encoding the thymidine kinase gene
to cell monolayers in culture. Expression of the exogenous
20 gene was detected in the target cells (Zhou et al., Journal
of Controlled Release, 1992, 19, 269-274).

    One major type of liposomal composition includes
phospholipids other than naturally-derived
phosphatidylcholine. Neutral liposome compositions, for
25 example, can be formed from dimyristoyl phosphatidylcholine
(DMPC) or dipalmitoyl phosphatidylcholine (DPPC). Anionic
liposome compositions generally are formed from dimyristoyl
phosphatidylglycerol, while anionic fusogenic liposomes are
formed primarily from dioleoyl phosphatidylethanolamine
30 (DOPE). Another type of liposomal composition is formed
from phosphatidylcholine (PC) such as, for example, soybean
PC, and egg PC. Another type is formed from mixtures of
phospholipid and/or phosphatidylcholine and/or cholesterol.

    Several studies have assessed the topical delivery of
35 liposomal drug formulations to the skin. Application of

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liposomes containing interferon to guinea pig skin resulted
in a reduction of skin herpes sores while delivery of
interferon via other means (e.g. as a solution or as an
emulsion) were ineffective (Weiner et al., Journal of Drug
5   Targeting, 1992, 2, 405-410). Further, an additional study
tested the efficacy of interferon administered as part of a
liposomal formulation to the administration of interferon
using an aqueous system, and concluded that the liposomal
formulation was superior to aqueous administration (du
10   Plessis et al., Antiviral Research, 1992, 18, 259-265).

Non-ionic liposomal systems have also been examined to
determine their utility in the delivery of drugs to the
skin, in particular systems comprising non-ionic surfactant
and cholesterol. Non-ionic liposomal formulations
15   comprising Novasome™ I (glyceryl
dilaurate/cholesterol/polyoxyethylene-10-stearyl ether) and
Novasome™ II (glyceryl distearate/
cholesterol/polyoxyethylene-10-stearyl ether) were used to
deliver cyclosporin-A into the dermis of mouse skin.
20   Results indicated that such non-ionic liposomal systems
were effective in facilitating the deposition of
cyclosporin-A into different layers of the skin (Hu et al.
S.T.P.Pharma. Sci., 1994, 4, 6, 466).

Liposomes also include "sterically stabilized"
25   liposomes, a term which, as used herein, refers to
liposomes comprising one or more specialized lipids that,
when incorporated into liposomes, result in enhanced
circulation lifetimes relative to liposomes lacking such
specialized lipids. Examples of sterically stabilized
30   liposomes are those in which part of the vesicle-forming
lipid portion of the liposome (A) comprises one or more
glycolipids, such as monosialoganglioside G_{M1}, or (B) is
derivatized with one or more hydrophilic polymers, such as
a polyethylene glycol (PEG) moiety. While not wishing to
35   be bound by any particular theory, it is thought in the art

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that, at least for sterically stabilized liposomes
containing gangliosides, sphingomyelin, or PEG-derivatized
lipids, the enhanced circulation half-life of these
sterically stabilized liposomes derives from a reduced
5   uptake into cells of the reticuloendothelial system (RES)
(Allen *et al.*, *FEBS Letters*, *1987*, *223*, 42; Wu *et al.*,
Cancer Research, **1993**, *53*, 3765).   Various liposomes
comprising one or more glycolipids are known in the art.
Papahadjopoulos *et al.* (*Ann. N.Y. Acad. Sci.*, **1987**, *507*,
10   64) reported the ability of monosialoganglioside G_{M1},
galactocerebroside sulfate and phosphatidylinositol to
improve blood half-lives of liposomes. These findings were
expounded upon by Gabizon *et al.* (*Proc. Natl. Acad. Sci.*
U.S.A., **1988**, *85*, 6949). U.S. Patent No. 4,837,028 and WO
15   88/04924, both to Allen *et al.*, disclose liposomes
comprising (1) sphingomyelin and (2) the ganglioside G_{M1} or a
galactocerebroside sulfate ester. U.S. Patent No.
5,543,152 (Webb *et al.*) discloses liposomes comprising
sphingomyelin. Liposomes comprising 1,2-*sn*-
20   dimyristoylphosphatidylcholine are disclosed in WO 97/13499
(Lim *et al.*).

Many liposomes comprising lipids derivatized with one
or more hydrophilic polymers, and methods of preparation
thereof, are known in the art. Sunamoto *et al.* (*Bull.*
25   *Chem. Soc. Jpn.*, **1980**, *53*, 2778) described liposomes
comprising a nonionic detergent, $2C_{12}15G$, that contains a
PEG moiety. Illum *et al.* (*FEBS Lett.*, **1984**, *167*, 79) noted
that hydrophilic coating of polystyrene particles with
polymeric glycols results in significantly enhanced blood
30   half-lives. Synthetic phospholipids modified by the
attachment of carboxylic groups of polyalkylene glycols
(*e.g.*, PEG) are described by Sears (U.S. Patent Nos.
4,426,330 and 4,534,899). Klibanov *et al.* (*FEBS Lett.*,
1990, *268*, 235) described experiments demonstrating that

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liposomes comprising phosphatidylethanolamine (PE) derivatized with PEG or PEG stearate have significant increases in blood circulation half-lives. Blume *et al.* (*Biochimica et Biophysica Acta*, **1990**, *1029*, 91) extended such observations to other PEG-derivatized phospholipids, *e.g.*, DSPE-PEG, formed from the combination of distearoylphosphatidylethanolamine (DSPE) and PEG. Liposomes having covalently bound PEG moieties on their external surface are described in European Patent No. EP 0 445 131 B1 and WO 90/04384 to Fisher. Liposome compositions containing 1-20 mole percent of PE derivatized with PEG, and methods of use thereof, are described by Woodle *et al.* (U.S. Patent Nos. 5,013,556 and 5,356,633) and Martin *et al.* (U.S. Patent No. 5,213,804 and European Patent No. EP 0 496 813 B1). Liposomes comprising a number of other lipid-polymer conjugates are disclosed in WO 91/05545 and U.S. Patent No. 5,225,212 (both to Martin *et al.*) and in WO 94/20073 (Zalipsky *et al.*) Liposomes comprising PEG-modified ceramide lipids are described in WO 96/10391 (Choi *et al.*). U.S. Patent Nos. 5,540,935 (Miyazaki *et al.*) and 5,556,948 (Tagawa *et al.*) describe PEG-containing liposomes that can be further derivatized with functional moieties on their surfaces.

A limited number of liposomes comprising nucleic acids are known in the art. WO 96/40062 to Thierry *et al.* discloses methods for encapsulating high molecular weight nucleic acids in liposomes. U.S. Patent No. 5,264,221 to Tagawa *et al.* discloses protein-bonded liposomes and asserts that the contents of such liposomes may include an antisense RNA. U.S. Patent No. 5,665,710 to Rahman *et al.* describes certain methods of encapsulating oligodeoxynucleotides in liposomes. WO 97/04787 to Love *et al.* discloses liposomes comprising antisense oligonucleotides targeted to the raf gene.

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Transfersomes are yet another type of liposomes, and
are highly deformable lipid aggregates which are attractive
candidates for drug delivery vehicles. Transfersomes may
be described as lipid droplets which are so highly

5   deformable that they are easily able to penetrate through
pores which are smaller than the droplet. Transfersomes
are adaptable to the environment in which they are used,
e.g. they are self-optimizing (adaptive to the shape of
pores in the skin), self-repairing, frequently reach their

10   targets without fragmenting, and often self-loading. To
make transfersomes it is possible to add surface edge-
activators, usually surfactants, to a standard liposomal
composition. Transfersomes have been used to deliver serum
albumin to the skin. The transfersome-mediated delivery of

15   serum albumin has been shown to be as effective as
subcutaneous injection of a solution containing serum
albumin.

Surfactants find wide application in formulations such
as emulsions (including microemulsions) and liposomes. The

20   most common way of classifying and ranking the properties
of the many different types of surfactants, both natural
and synthetic, is by the use of the hydrophile/lipophile
balance (HLB). The nature of the hydrophilic group (also
known as the "head") provides the most useful means for

25   categorizing the different surfactants used in formulations
(Rieger, in Pharmaceutical Dosage Forms, Marcel Dekker,
Inc., New York, NY, 1988, p. 285).

If the surfactant molecule is not ionized, it is
classified as a nonionic surfactant. Nonionic surfactants

30   find wide application in pharmaceutical and cosmetic
products and are usable over a wide range of pH values. In
general their HLB values range from 2 to about 18 depending
on their structure. Nonionic surfactants include nonionic
esters such as ethylene glycol esters, propylene glycol

35   esters, glyceryl esters, polyglyceryl esters, sorbitan

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esters, sucrose esters, and ethoxylated esters. Nonionic alkanolamides and ethers such as fatty alcohol ethoxylates, propoxylated alcohols, and ethoxylated/propoxylated block polymers are also included in this class. The
5   polyoxyethylene surfactants are the most popular members of the nonionic surfactant class.

If the surfactant molecule carries a negative charge when it is dissolved or dispersed in water, the surfactant is classified as anionic. Anionic surfactants include
10 carboxylates such as soaps, acyl lactylates, acyl amides of amino acids, esters of sulfuric acid such as alkyl sulfates and ethoxylated alkyl sulfates, sulfonates such as alkyl benzene sulfonates, acyl isethionates, acyl taurates and sulfosuccinates, and phosphates. The most important
15 members of the anionic surfactant class are the alkyl sulfates and the soaps.

If the surfactant molecule carries a positive charge when it is dissolved or dispersed in water, the surfactant is classified as cationic. Cationic surfactants include
20 quaternary ammonium salts and ethoxylated amines. The quaternary ammonium salts are the most used members of this class.

If the surfactant molecule has the ability to carry either a positive or negative charge, the surfactant is
25 classified as amphoteric. Amphoteric surfactants include acrylic acid derivatives, substituted alkylamides, N-alkylbetaines and phosphatides.

The use of surfactants in drug products, formulations and in emulsions has been reviewed (Rieger, in
30 *Pharmaceutical Dosage Forms*, Marcel Dekker, Inc., New York, NY, **1988**, p. 285).

Penetration Enhancers

In one embodiment, the present invention employs
35 various penetration enhancers to effect the efficient

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delivery of nucleic acids, particularly oligonucleotides, to the skin of animals. Most drugs are present in solution in both ionized and nonionized forms. However, usually only lipid soluble or lipophilic drugs readily cross cell
5   membranes. It has been discovered that even non-lipophilic drugs may cross cell membranes if the membrane to be crossed is treated with a penetration enhancer. In addition to aiding the diffusion of non-lipophilic drugs across cell membranes, penetration enhancers also enhance
10   the permeability of lipophilic drugs.

Penetration enhancers may be classified as belonging to one of five broad categories, *i.e.*, surfactants, fatty acids, bile salts, chelating agents, and non-chelating non-surfactants (Lee *et al.*, *Critical Reviews in Therapeutic*
15   *Drug Carrier Systems*, **1991**, p.92). Each of the above mentioned classes of penetration enhancers are described below in greater detail.

Surfactants: In connection with the present invention,
20   surfactants (or "surface-active agents") are chemical entities which, when dissolved in an aqueous solution, reduce the surface tension of the solution or the interfacial tension between the aqueous solution and another liquid, with the result that absorption of
25   oligonucleotides through the mucosa is enhanced. In addition to bile salts and fatty acids, these penetration enhancers include, for example, sodium lauryl sulfate, polyoxyethylene-9-lauryl ether and polyoxyethylene-20-cetyl ether) (Lee *et al.*, *Critical Reviews in Therapeutic Drug*
30   *Carrier Systems*, **1991**, p.92); and perfluorochemical emulsions, such as FC-43. Takahashi *et al.*, *J. Pharm. Pharmacol.*, **1988**, *40*, 252).

Fatty acids: Various fatty acids and their derivatives which act as penetration enhancers include, for example,

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oleic acid, lauric acid, capric acid (n-decanoic acid),
myristic acid, palmitic acid, stearic acid, linoleic acid,
linolenic acid, dicaprate, tricaprate, monoolein (1-
monooleoyl-rac-glycerol), dilaurin, caprylic acid,

5   arachidonic acid, glycerol 1-monocaprate, 1-
dodecylazacycloheptan-2-one, acylcarnitines, acylcholines,
C_{1-10} alkyl esters thereof (e.g., methyl, isopropyl and t-
butyl), and mono- and di-glycerides thereof (i.e., oleate,
laurate, caprate, myristate, palmitate, stearate,

10   linoleate, etc.) (Lee et al., Critical Reviews in
Therapeutic Drug Carrier Systems, 1991, p.92; Muranishi,
Critical Reviews in Therapeutic Drug Carrier Systems, 1990,
7, 1-33; El Hariri et al., J. Pharm. Pharmacol., 1992, 44,
651-654).

15

     Bile salts: The physiological role of bile includes
the facilitation of dispersion and absorption of lipids and
fat-soluble vitamins (Brunton, Chapter 38 in: Goodman &
Gilman's The Pharmacological Basis of Therapeutics, 9th

20   Ed., Hardman et al. Eds., McGraw-Hill, New York, 1996, pp.
934-935). Various natural bile salts, and their synthetic
derivatives, act as penetration enhancers. Thus the term
"bile salts" includes any of the naturally occurring
components of bile as well as any of their synthetic

25   derivatives.   The bile salts of the invention include, for
example, cholic acid (or its pharmaceutically acceptable
sodium salt, sodium cholate), dehydrocholic acid (sodium
dehydrocholate), deoxycholic acid (sodium deoxycholate),
glucholic acid (sodium glucholate), glycholic acid (sodium

30   glycocholate), glycodeoxycholic acid (sodium
glycodeoxycholate), taurocholic acid (sodium taurocholate),
taurodeoxycholic acid (sodium taurodeoxycholate),
chenodeoxycholic acid (sodium chenodeoxycholate),
ursodeoxycholic acid (UDCA), sodium tauro-24,25-dihydro-

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fusidate (STDHF), sodium glycodihydrofusidate and
polyoxyethylene-9-lauryl ether (POE) (Lee *et al.*, *Critical
Reviews in Therapeutic Drug Carrier Systems*, 1991, page 92;
Swinyard, Chapter 39 *In: Remington's Pharmaceutical*
5   *Sciences*, 18th Ed., Gennaro, ed., Mack Publishing Co.,
Easton, PA, 1990, pages 782-783; Muranishi, *Critical
Reviews in Therapeutic Drug Carrier Systems*, 1990, 7, 1-33;
Yamamoto *et al.*, *J. Pharm. Exp. Ther.*, 1992, *263*, 25;
Yamashita *et al.*, *J. Pharm. Sci.*, 1990, *79*, 579-583).
10

Chelating Agents: Chelating agents, as used in
connection with the present invention, can be defined as
compounds that remove metallic ions from solution by
forming complexes therewith, with the result that
15   absorption of oligonucleotides through the mucosa is
enhanced. With regards to their use as penetration
enhancers in the present invention, chelating agents have
the added advantage of also serving as DNase inhibitors, as
most characterized DNA nucleases require a divalent metal
20   ion for catalysis and are thus inhibited by chelating
agents (Jarrett, *J. Chromatogr.*, 1993, *618*, 315-339).
Chelating agents of the invention include but are not
limited to disodium ethylenediaminetetraacetate (EDTA),
citric acid, salicylates (*e.g.*, sodium salicylate, 5-
25   methoxysalicylate and homovanilate), *N*-acyl derivatives of
collagen, laureth-9 and *N*-amino acyl derivatives of beta-
diketones (enamines)(Lee *et al.*, *Critical Reviews in
Therapeutic Drug Carrier Systems*, 1991, page 92; Muranishi,
Critical Reviews in Therapeutic Drug Carrier Systems, 1990,
30   7, 1-33; Buur *et al.*, *J. Control Rel.*, 1990, *14*, 43-51).

Non-chelating non-surfactants: As used herein, non-
chelating non-surfactant penetration enhancing compounds
can be defined as compounds that demonstrate insignificant

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activity as chelating agents or as surfactants but that
nonetheless enhance absorption of oligonucleotides through
the alimentary mucosa (Muranishi, *Critical Reviews in
Therapeutic Drug Carrier Systems*, **1990**, *7*, 1-33). This
5   class of penetration enhancers include, for example,
unsaturated cyclic ureas, 1-alkyl- and 1-alkenylazacyclo-
alkanone derivatives (Lee *et al.*, *Critical Reviews in
Therapeutic Drug Carrier Systems*, **1991**, page 92); and non-
steroidal anti-inflammatory agents such as diclofenac
10   sodium, indomethacin and phenylbutazone (Yamashita *et al.*,
J. Pharm. Pharmacol., **1987**, *39*, 621-626).

Agents that enhance uptake of oligonucleotides at the
cellular level may also be added to the pharmaceutical and
other compositions of the present invention. For example,
15   cationic lipids, such as lipofectin (Junichi *et al*, U.S.
Patent No. 5,705,188), cationic glycerol derivatives, and
polycationic molecules, such as polylysine (Lollo *et al.*,
PCT Application WO 97/30731), are also known to enhance the
cellular uptake of oligonucleotides.
20   Other agents may be utilized to enhance the
penetration of the administered nucleic acids, including
glycols such as ethylene glycol and propylene glycol,
pyrrols such as 2-pyrrol, azones, and terpenes such as
limonene and menthone.
25

Carriers

Certain compositions of the present invention also
incorporate carrier compounds in the formulation. As used
herein, "carrier compound" or "carrier" can refer to a
30   nucleic acid, or analog thereof, which is inert (*i.e.*, does
not possess biological activity *per se*) but is recognized
as a nucleic acid by *in vivo* processes that reduce the
bioavailability of a nucleic acid having biological
activity by, for example, degrading the biologically active

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nucleic acid or promoting its removal from circulation.
The coadministration of a nucleic acid and a carrier
compound, typically with an excess of the latter substance,
can result in a substantial reduction of the amount of

5   nucleic acid recovered in the liver, kidney or other
extracirculatory reservoirs, presumably due to competition
between the carrier compound and the nucleic acid for a
common receptor. For example, the recovery of a partially
phosphorothioate oligonucleotide in hepatic tissue can be

10   reduced when it is coadministered with polyinosinic acid,
dextran sulfate, polycytidic acid or 4-acetamido-
4'isothiocyano-stilbene-2,2'-disulfonic acid (Miyao *et al.*,
Antisense Res. Dev., 1995, *5*, 115-121; Takakura *et al.*,
Antisense & Nucl. Acid Drug Dev., 1996, *6*, 177-183).

15

Excipients
        In contrast to a carrier compound, a "pharmaceutical
carrier" or "excipient" is a pharmaceutically acceptable
solvent, suspending agent or any other pharmacologically

20   inert vehicle for delivering one or more nucleic acids to
an animal. The excipient may be liquid or solid and is
selected, with the planned manner of administration in
mind, so as to provide for the desired bulk, consistency,
etc., when combined with a nucleic acid and the other

25   components of a given pharmaceutical composition. Typical
pharmaceutical carriers include, but are not limited to,
binding agents (*e.g.*, pregelatinized maize starch,
polyvinylpyrrolidone or hydroxypropyl methylcellulose,
etc.); fillers (*e.g.*, lactose and other sugars,

30   microcrystalline cellulose, pectin, gelatin, calcium
sulfate, ethyl cellulose, polyacrylates or calcium hydrogen
phosphate, *etc.*); lubricants (*e.g.*, magnesium stearate,
talc, silica, colloidal silicon dioxide, stearic acid,
metallic stearates, hydrogenated vegetable oils, corn

35   starch, polyethylene glycols, sodium benzoate, sodium

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acetate, *etc.*); disintegrants (*e.g.*, starch, sodium starch glycolate, *etc.*); and wetting agents (*e.g.*, sodium lauryl sulphate, *etc.*).

Pharmaceutically acceptable organic or inorganic
5   excipient suitable for non-parenteral administration which do not deleteriously react with nucleic acids can also be used to formulate the compositions of the present invention. Suitable pharmaceutically acceptable carriers include, but are not limited to, water, salt solutions,
10   alcohols, polyethylene glycols, gelatin, lactose, amylose, magnesium stearate, talc, silicic acid, viscous paraffin, hydroxymethylcellulose, polyvinylpyrrolidone and the like.

Formulations for topical administration of nucleic acids may include sterile and non-sterile aqueous
15   solutions, non-aqueous solutions in common solvents such as alcohols, or solutions of the nucleic acids in liquid or solid oil bases. The solutions may also contain buffers, diluents and other suitable additives. Pharmaceutically acceptable organic or inorganic excipients suitable for
20   non-parenteral administration which do not deleteriously react with nucleic acids can be used.

Suitable pharmaceutically acceptable excipients include, but are not limited to, water, salt solutions, alcohol, polyethylene glycols, gelatin, lactose, amylose,
25   magnesium stearate, talc, silicic acid, viscous paraffin, hydroxymethylcellulose, polyvinylpyrrolidone and the like.

Other Components

The compositions of the present invention may
30   additionally contain other adjunct components conventionally found in pharmaceutical compositions, at their art-established usage levels. Thus, for example, the compositions may contain additional, compatible, pharmaceutically-active materials such as, for example,
35   antipruritics, astringents, local anesthetics or

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anti-inflammatory agents, or may contain additional
materials useful in physically formulating various dosage
forms of the compositions of the present invention, such as
dyes, flavoring agents, preservatives, antioxidants,

5   opacifiers, thickening agents and stabilizers. However,
such materials, when added, should not unduly interfere
with the biological activities of the components of the
compositions of the present invention. The formulations
can be sterilized and, if desired, mixed with auxiliary

10 agents, e.g., lubricants, preservatives, stabilizers,
wetting agents, emulsifiers, salts for influencing osmotic
pressure, buffers, colorings, flavorings and/or aromatic
substances and the like which do not deleteriously interact
with the nucleic acid(s) of the formulation.

15   Aqueous suspensions may contain substances which
increase the viscosity of the suspension including, for
example, sodium carboxymethylcellulose, sorbitol and/or
dextran. The suspension may also contain stabilizers.

            Certain embodiments of the invention provide
20 pharmaceutical compositions containing (a) one or more
antisense compounds and (b) one or more other
chemotherapeutic agents which function by a non-antisense
mechanism. Examples of such chemotherapeutic agents
include, but are not limited to, anticancer drugs such as

25 daunorubicin, dactinomycin, doxorubicin, bleomycin,
mitomycin, nitrogen mustard, chlorambucil, melphalan,
cyclophosphamide, 6-mercaptopurine, 6-thioguanine,
cytarabine (CA), 5-fluorouracil (5-FU), floxuridine
(5-FUdR), methotrexate (MTX), colchicine, vincristine,

30 vinblastine, etoposide, teniposide, cisplatin and
diethylstilbestrol (DES). See, generally, The Merck Manual
of Diagnosis and Therapy, 15th Ed., Berkow et al., eds.,
1987, Rahway, N.J., pages 1206-1228). Anti-inflammatory
drugs, including but not limited to nonsteroidal anti-

35 inflammatory drugs and corticosteroids, and antiviral

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drugs, including but not limited to ribivirin, vidarabine, acyclovir and ganciclovir, may also be combined in compositions of the invention. See, generally, *The Merck Manual of Diagnosis and Therapy*, 15th Ed., Berkow et al.,

5   eds., **1987**, Rahway, N.J., pages 2499-2506 and 46-49, respectively). Other non-antisense chemotherapeutic agents are also within the scope of this invention. Two or more combined compounds may be used together or sequentially.

In another related embodiment, compositions of the

10 invention may contain one or more antisense compounds, particularly oligonucleotides, targeted to a first nucleic acid and one or more additional antisense compounds targeted to a second nucleic acid target. Numerous examples of antisense compounds are known in the art. Two or more

15 combined compounds may be used together or sequentially.

The formulation of therapeutic compositions and their subsequent administration is believed to be within the skill of those in the art. Dosing is dependent on severity and responsiveness of the disease state to be treated, with

20 the course of treatment lasting from several days to several months, or until a cure is effected or a diminution of the disease state is achieved. Optimal dosing schedules can be calculated from measurements of drug accumulation in the body of the patient. Persons of ordinary skill can

25 easily determine optimum dosages, dosing methodologies and repetition rates. Optimum dosages may vary depending on the relative potency of individual oligonucleotides, and can generally be estimated based on EC_{50}s found to be effective in in vitro and in vivo animal models. In

30 general, dosage is from 0.01 ug to 100 g per kg of body weight, and may be given once or more daily, weekly, monthly or yearly, or even once every 2 to 20 years. Persons of ordinary skill in the art can easily estimate repetition rates for dosing based on measured residence

35 times and concentrations of the drug in bodily fluids or

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tissues. Following successful treatment, it may be desirable to have the patient undergo maintenance therapy to prevent the recurrence of the disease state, wherein the oligonucleotide is administered in maintenance doses,

5   ranging from 0.01 ug to 100 g per kg of body weight, once or more daily, to once every 20 years.

While the present invention has been described with specificity in accordance with certain of its preferred embodiments, the following examples serve only to

10 illustrate the invention and are not intended to limit the same.

EXAMPLES

Example 1

15 **Nucleoside Phosphoramidites for Oligonucleotide Synthesis**
Deoxy and 2'-alkoxy amidites

2'-Deoxy and 2'-methoxy beta-cyanoethyldiisopropyl phosphoramidites were purchased from commercial sources (e.g. Chemgenes, Needham MA or Glen Research, Inc. Sterling

20 VA). Other 2'-O-alkoxy substituted nucleoside amidites are prepared as described in U.S. Patent 5,506,351, herein incorporated by reference. For oligonucleotides synthesized using 2'-alkoxy amidites, the standard cycle for unmodified oligonucleotides was utilized, except the

25 wait step after pulse delivery of tetrazole and base was increased to 360 seconds.

Oligonucleotides containing 5-methyl-2'-deoxycytidine (5-Me-C) nucleotides were synthesized according to published methods [Sanghvi, et. al., *Nucleic Acids*

30 *Research*, **1993**, *21*, 3197-3203] using commercially available phosphoramidites (Glen Research, Sterling VA or ChemGenes, Needham MA).

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2'-Fluoro amidites

2'-Fluorodeoxyadenosine amidites

2'-fluoro oligonucleotides were synthesized as
described previously [Kawasaki, et. al., *J. Med. Chem.*,
1993, *36*, 831-841] and United States patent 5,670,633,
herein incorporated by reference. Briefly, the protected
nucleoside N6-benzoyl-2'-deoxy-2'-fluoroadenosine was
synthesized utilizing commercially available 9-beta-D-
arabinofuranosyladenine as starting material and by
modifying literature procedures whereby the 2'-alpha-fluoro
atom is introduced by a S_N2-displacement of a 2'-beta-trityl
group. Thus N6-benzoyl-9-beta-D-arabinofuranosyladenine
was selectively protected in moderate yield as the 3',5'-
ditetrahydropyranyl (THP) intermediate. Deprotection of
the THP and N6-benzoyl groups was accomplished using
standard methodologies and standard methods were used to
obtain the 5'-dimethoxytrityl-(DMT) and 5'-DMT-3'-
phosphoramidite intermediates.

2'-Fluorodeoxyguanosine

The synthesis of 2'-deoxy-2'-fluoroguanosine was
accomplished using tetraisopropyldisiloxanyl (TPDS)
protected 9-beta-D-arabinofuranosylguanine as starting
material, and conversion to the intermediate diisobutyryl-
arabinofuranosylguanosine. Deprotection of the TPDS group
was followed by protection of the hydroxyl group with THP
to give diisobutyryl di-THP protected
arabinofuranosylguanine. Selective O-deacylation and
triflation was followed by treatment of the crude product
with fluoride, then deprotection of the THP groups.
Standard methodologies were used to obtain the 5'-DMT- and
5'-DMT-3'-phosphoramidites.

2'-Fluorouridine

Synthesis of 2'-deoxy-2'-fluorouridine was
accomplished by the modification of a literature procedure

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in which 2,2'-anhydro-1-beta-D-arabinofuranosyluracil was
treated with 70% hydrogen fluoride-pyridine. Standard
procedures were used to obtain the 5'-DMT and 5'-DMT-
3'phosphoramidites.

5   **2'-Fluorodeoxycytidine**

2'-deoxy-2'-fluorocytidine was synthesized via
amination of 2'-deoxy-2'-fluorouridine, followed by
selective protection to give N4-benzoyl-2'-deoxy-2'-
fluorocytidine. Standard procedures were used to obtain

10   the 5'-DMT and 5'-DMT-3'phosphoramidites.

2'-O-(2-Methoxyethyl) modified amidites

2'-O-Methoxyethyl-substituted nucleoside amidites are
prepared as follows, or alternatively, as per the methods
of Martin, P., *Helvetica Chimica Acta*, **1995**, *78*, 486-504.

15   **2,2'-Anhydro[1-(beta-D-arabinofuranosyl)-5-
         methyluridine]**

5-Methyluridine (ribosylthymine, commercially
available through Yamasa, Choshi, Japan) (72.0 g, 0.279 M),
diphenylcarbonate (90.0 g, 0.420 M) and sodium bicarbonate

20   (2.0 g, 0.024 M) were added to DMF (300 mL). The mixture
was heated to reflux, with stirring, allowing the evolved
carbon dioxide gas to be released in a controlled manner.
After 1 hour, the slightly darkened solution was
concentrated under reduced pressure. The resulting syrup

25   was poured into diethylether (2.5 L), with stirring. The
product formed a gum. The ether was decanted and the
residue was dissolved in a minimum amount of methanol (ca.
400 mL). The solution was poured into fresh ether (2.5 L)
to yield a stiff gum. The ether was decanted and the gum

30   was dried in a vacuum oven (60°C at 1 mm Hg for 24 h) to
give a solid that was crushed to a light tan powder (57 g,
85% crude yield). The NMR spectrum was consistent with the
structure, contaminated with phenol as its sodium salt (ca.
5%). The material was used as is for further reactions (or

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it can be purified further by column chromatography using a
gradient of methanol in ethyl acetate (10-25%) to give a
white solid, mp 222-4°C).

2'-O-Methoxyethyl-5-methyluridine

2,2'-Anhydro-5-methyluridine (195 g, 0.81 M), tris(2-
methoxyethyl)borate (231 g, 0.98 M) and 2-methoxyethanol
(1.2 L) were added to a 2 L stainless steel pressure vessel
and placed in a pre-heated oil bath at 160°C. After heating
for 48 hours at 155-160°C, the vessel was opened and the
solution evaporated to dryness and triturated with MeOH
(200 mL). The residue was suspended in hot acetone (1 L).
The insoluble salts were filtered, washed with acetone (150
mL) and the filtrate evaporated. The residue (280 g) was
dissolved in CH_3CN (600 mL) and evaporated. A silica gel
column (3 kg) was packed in CH_2Cl_2/acetone/MeOH (20:5:3)
containing 0.5% Et_3NH. The residue was dissolved in CH_2Cl_2
(250 mL) and adsorbed onto silica (150 g) prior to loading
onto the column. The product was eluted with the packing
solvent to give 160 g (63%) of product. Additional
material was obtained by reworking impure fractions.

2'-O-Methoxyethyl-5'-O-dimethoxytrityl-5-methyluridine

2'-O-Methoxyethyl-5-methyluridine (160 g, 0.506 M) was
co-evaporated with pyridine (250 mL) and the dried residue
dissolved in pyridine (1.3 L). A first aliquot of
dimethoxytrityl chloride (94.3 g, 0.278 M) was added and
the mixture stirred at room temperature for one hour. A
second aliquot of dimethoxytrityl chloride (94.3 g, 0.278
M) was added and the reaction stirred for an additional one
hour. Methanol (170 mL) was then added to stop the
reaction. HPLC showed the presence of approximately 70%
product. The solvent was evaporated and triturated with
CH_3CN (200 mL). The residue was dissolved in $CHCl_3$ (1.5 L)
and extracted with 2x500 mL of saturated $NaHCO_3$ and 2x500 mL
of saturated NaCl. The organic phase was dried over Na_2SO_4,
filtered and evaporated. 275 g of residue was obtained.

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The residue was purified on a 3.5 kg silica gel column, packed and eluted with EtOAc/hexane/acetone (5:5:1) containing 0.5% Et₃NH. The pure fractions were evaporated to give 164 g of product. Approximately 20 g additional

5 was obtained from the impure fractions to give a total yield of 183 g (57%).

3'-O-Acetyl-2'-O-methoxyethyl-5'-O-dimethoxytrityl-5-methyluridine

2'-O-Methoxyethyl-5'-O-dimethoxytrityl-5-methyluridine

10 (106 g, 0.167 M), DMF/pyridine (750 mL of a 3:1 mixture prepared from 562 mL of DMF and 188 mL of pyridine) and acetic anhydride (24.38 mL, 0.258 M) were combined and stirred at room temperature for 24 hours. The reaction was monitored by TLC by first quenching the TLC sample with the

15 addition of MeOH. Upon completion of the reaction, as judged by TLC, MeOH (50 mL) was added and the mixture evaporated at 35°C. The residue was dissolved in CHCl₃ (800 mL) and extracted with 2x200 mL of saturated sodium bicarbonate and 2x200 mL of saturated NaCl. The water

20 layers were back extracted with 200 mL of CHCl₃. The combined organics were dried with sodium sulfate and evaporated to give 122 g of residue (approx. 90% product). The residue was purified on a 3.5 kg silica gel column and eluted using EtOAc/hexane(4:1). Pure product fractions were

25 evaporated to yield 96 g (84%). An additional 1.5 g was recovered from later fractions.

3'-O-Acetyl-2'-O-methoxyethyl-5'-O-dimethoxytrityl-5-methyl-4-triazoleuridine

A first solution was prepared by dissolving 3'-O-

30 acetyl-2'-O-methoxyethyl-5'-O-dimethoxytrityl-5-methyluridine (96 g, 0.144 M) in CH₃CN (700 mL) and set aside. Triethylamine (189 mL, 1.44 M) was added to a solution of triazole (90 g, 1.3 M) in CH₃CN (1 L), cooled to -5°C and stirred for 0.5 h using an overhead stirrer. POCl₃

35 was added dropwise, over a 30 minute period, to the stirred

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solution maintained at 0-10°C, and the resulting mixture
stirred for an additional 2 hours. The first solution was
added dropwise, over a 45 minute period, to the latter
solution. The resulting reaction mixture was stored

5   overnight in a cold room. Salts were filtered from the
reaction mixture and the solution was evaporated. The
residue was dissolved in EtOAc (1 L) and the insoluble
solids were removed by filtration. The filtrate was washed
with 1x300 mL of NaHCO₃ and 2x300 mL of saturated NaCl,

10   dried over sodium sulfate and evaporated. The residue was
triturated with EtOAc to give the title compound.

2'-O-Methoxyethyl-5'-O-dimethoxytrityl-5-

methylcytidine

A solution of 3'-O-acetyl-2'-O-methoxyethyl-5'-O-

15   dimethoxytrityl-5-methyl-4-triazoleuridine (103 g, 0.141 M)
in dioxane (500 mL) and NH₄OH (30 mL) was stirred at room
temperature for 2 hours. The dioxane solution was
evaporated and the residue azeotroped with MeOH (2x200 mL).
The residue was dissolved in MeOH (300 mL) and transferred

20   to a 2 liter stainless steel pressure vessel. MeOH (400
mL) saturated with NH₃ gas was added and the vessel heated
to 100°C for 2 hours (TLC showed complete conversion). The
vessel contents were evaporated to dryness and the residue
was dissolved in EtOAc (500 mL) and washed once with

25   saturated NaCl (200 mL). The organics were dried over
sodium sulfate and the solvent was evaporated to give 85 g
(95%) of the title compound.

N4-Benzoyl-2'-O-methoxyethyl-5'-O-dimethoxytrityl-5-

methylcytidine

30   2'-O-Methoxyethyl-5'-O-dimethoxytrityl-5-methyl-
cytidine (85 g, 0.134 M) was dissolved in DMF (800 mL) and
benzoic anhydride (37.2 g, 0.165 M) was added with
stirring. After stirring for 3 hours, TLC showed the
reaction to be approximately 95% complete. The solvent was

35   evaporated and the residue azeotroped with MeOH (200 mL).

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The residue was dissolved in CHCl₃ (700 mL) and extracted with saturated NaHCO₃ (2x300 mL) and saturated NaCl (2x300 mL), dried over MgSO₄ and evaporated to give a residue (96 g). The residue was chromatographed on a 1.5 kg silica column using EtOAc/hexane (1:1) containing 0.5% Et₃NH as the eluting solvent. The pure product fractions were evaporated to give 90 g (90%) of the title compound.

N4-Benzoyl-2'-O-methoxyethyl-5'-O-dimethoxytrityl-5-methylcytidine-3'-amidite

N4-Benzoyl-2'-O-methoxyethyl-5'-O-dimethoxytrityl-5-methylcytidine (74 g, 0.10 M) was dissolved in CH₂Cl₂ (1 L). Tetrazole diisopropylamine (7.1 g) and 2-cyanoethoxy-tetra-(isopropyl)phosphite (40.5 mL, 0.123 M) were added with stirring, under a nitrogen atmosphere. The resulting mixture was stirred for 20 hours at room temperature (TLC showed the reaction to be 95% complete). The reaction mixture was extracted with saturated NaHCO₃ (1x300 mL) and saturated NaCl (3x300 mL). The aqueous washes were back-extracted with CH₂Cl₂ (300 mL), and the extracts were combined, dried over MgSO₄ and concentrated. The residue obtained was chromatographed on a 1.5 kg silica column using EtOAc/hexane (3:1) as the eluting solvent. The pure fractions were combined to give 90.6 g (87%) of the title compound.

2'-O-(Aminooxyethyl) nucleoside amidites and 2'-O-(dimethylaminooxyethyl) nucleoside amidites

2'-(Dimethylaminooxyethoxy) nucleoside amidites

2'-(Dimethylaminooxyethoxy) nucleoside amidites [also known in the art as 2'-O-(dimethylaminooxyethyl) nucleoside amidites] are prepared as described in the following paragraphs. Adenosine, cytidine and guanosine nucleoside amidites are prepared similarly to the thymidine (5-methyluridine) except the exocyclic amines are protected with a benzoyl moiety in the case of adenosine and cytidine

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and with isobutyryl in the case of guanosine.

5'-O-tert-Butyldiphenylsilyl-O^2-2'-anhydro-5-methyluridine

O^2-2'-anhydro-5-methyluridine (Pro. Bio. Sint., Varese,
5   Italy, 100.0g, 0.416 mmol), dimethylaminopyridine (0.66g,
0.013eq, 0.0054mmol) were dissolved in dry pyridine (500
ml) at ambient temperature under an argon atmosphere and
with mechanical stirring. tert-Butyldiphenylchlorosilane
(125.8g, 119.0mL, 1.1eq, 0.458mmol) was added in one
10   portion. The reaction was stirred for 16 h at ambient
temperature. TLC (Rf 0.22, ethyl acetate) indicated a
complete reaction. The solution was concentrated under
reduced pressure to a thick oil. This was partitioned
between dichloromethane (1 L) and saturated sodium
15   bicarbonate (2x1 L) and brine (1 L). The organic layer was
dried over sodium sulfate and concentrated under reduced
pressure to a thick oil. The oil was dissolved in a 1:1
mixture of ethyl acetate and ethyl ether (600mL) and the
solution was cooled to
20   -10°C. The resulting crystalline product was collected by
filtration, washed with ethyl ether (3x200 mL) and dried
(40°C, 1mm Hg, 24 h) to 149g (74.8%) of white solid. TLC
and NMR were consistent with pure product.

5'-O-tert-Butyldiphenylsilyl-2'-O-(2-hydroxyethyl)-5-methyluridine

In a 2 L stainless steel, unstirred pressure reactor
was added borane in tetrahydrofuran (1.0 M, 2.0 eq, 622
mL). In the fume hood and with manual stirring, ethylene
glycol (350 mL, excess) was added cautiously at first until
30   the evolution of hydrogen gas subsided. 5'-O-tert-
Butyldiphenylsilyl-O^2-2'-anhydro-5-methyluridine (149 g,
0.311 mol) and sodium bicarbonate (0.074 g, 0.003 eq) were
added with manual stirring. The reactor was sealed and
heated in an oil bath until an internal temperature of 160

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°C was reached and then maintained for 16 h (pressure < 100
psig). The reaction vessel was cooled to ambient and
opened. TLC (Rf 0.67 for desired product and Rf 0.82 for
ara-T side product, ethyl acetate) indicated about 70%

5   conversion to the product. In order to avoid additional
side product formation, the reaction was stopped,
concentrated under reduced pressure (10 to 1mm Hg) in a
warm water bath (40-100°C) with the more extreme conditions
used to remove the ethylene glycol. [Alternatively, once

10 the low boiling solvent is gone, the remaining solution can
be partitioned between ethyl acetate and water. The
product will be in the organic phase.] The residue was
purified by column chromatography (2kg silica gel, ethyl
acetate-hexanes gradient 1:1 to 4:1). The appropriate

15 fractions were combined, stripped and dried to product as a
white crisp foam (84g, 50%), contaminated starting material
(17.4g) and pure reusable starting material 20g. The yield
based on starting material less pure recovered starting
material was 58%. TLC and NMR were consistent with 99%

20 pure product.

     **2'-O-([2-phthalimidoxy)ethyl]-5'-t-butyldiphenylsilyl-
     5-methyluridine**

     5'-O-tert-Butyldiphenylsilyl-2'-O-(2-hydroxyethyl)-5-
methyluridine (20g, 36.98mmol) was mixed with

25 triphenylphosphine (11.63g, 44.36mmol) and N-
hydroxyphthalimide (7.24g, 44.36mmol). It was then dried
over P_2O_5 under high vacuum for two days at 40°C. The
reaction mixture was flushed with argon and dry THF
(369.8mL, Aldrich, sure seal bottle) was added to get a

30 clear solution. Diethyl-azodicarboxylate (6.98mL,
44.36mmol) was added dropwise to the reaction mixture. The
rate of addition is maintained such that resulting deep red
coloration is just discharged before adding the next drop.
After the addition was complete, the reaction was stirred

35 for 4 hrs. By that time TLC showed the completion of the

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reaction (ethylacetate:hexane, 60:40). The solvent was
evaporated in vacuum. Residue obtained was placed on a
flash column and eluted with ethyl acetate:hexane (60:40),
to get 2'-O-([2-phthalimidoxy)ethyl]-5'-t-
5   butyldiphenylsilyl-5-methyluridine as white foam (21.819 g,
86%).

5'-O-*tert*-butyldiphenylsilyl-2'-O-[(2-

formadoximinooxy)ethyl]-5-methyluridine

      2'-O-([2-phthalimidoxy)ethyl]-5'-t-butyldiphenylsilyl-
10   5-methyluridine (3.1g, 4.5mmol) was dissolved in dry CH_2Cl_2
(4.5mL) and methylhydrazine (300mL, 4.64mmol) was added
dropwise at -10°C to 0°C. After 1 h the mixture was
filtered, the filtrate was washed with ice cold CH_2Cl_2 and
the combined organic phase was washed with water, brine and
15   dried over anhydrous Na_2SO_4. The solution was concentrated
to get 2'-O-(aminooxyethyl) thymidine, which was then
dissolved in MeOH (67.5mL). To this formaldehyde (20%
aqueous solution, w/w, 1.1 eq.) was added and the resulting
mixture was strirred for 1 h. Solvent was removed under
20   vacuum; residue chromatographed to get 5'-O-*tert*-
butyldiphenylsilyl-2'-O-[(2-formadoximinooxy) ethyl]-5-
methyluridine as white foam (1.95 g, 78%).

        5'-O-*tert*-Butyldiphenylsilyl-2'-O-[N,N-

        dimethylaminooxyethyl]-5-methyluridine

25   5'-O-*tert*-butyldiphenylsilyl-2'-O-[(2-
formadoximinooxy)ethyl]-5-methyluridine (1.77g, 3.12mmol)
was dissolved in a solution of 1M pyridinium p-
toluenesulfonate (PPTS) in dry MeOH (30.6mL). Sodium
cyanoborohydride (0.39g, 6.13mmol) was added to this
30   solution at 10°C under inert atmosphere. The reaction
mixture was stirred for 10 minutes at 10°C. After that the
reaction vessel was removed from the ice bath and stirred
at room temperature for 2 h, the reaction monitored by TLC
(5% MeOH in CH_2Cl_2). Aqueous $NaHCO_3$ solution (5%, 10mL) was

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added and extracted with ethyl acetate (2x20mL). Ethyl
acetate phase was dried over anhydrous Na$_2$SO$_4$, evaporated to
dryness. Residue was dissolved in a solution of 1M PPTS in
MeOH (30.6mL). Formaldehyde (20% w/w, 30mL, 3.37mmol) was
5   added and the reaction mixture was stirred at room
temperature for 10 minutes. Reaction mixture cooled to 10°C
in an ice bath, sodium cyanoborohydride (0.39g, 6.13mmol)
was added and reaction mixture stirred at 10°C for 10
minutes. After 10 minutes, the reaction mixture was
10   removed from the ice bath and stirred at room temperature
for 2 hrs. To the reaction mixture 5% NaHCO$_3$ (25mL)
solution was added and extracted with ethyl acetate
(2x25mL). Ethyl acetate layer was dried over anhydrous
Na$_2$SO$_4$ and evaporated to dryness . The residue obtained was
15   purified by flash column chromatography and eluted with 5%
MeOH in CH$_2$Cl$_2$ to get 5'-O-*tert*-butyldiphenylsilyl-2'-O-
[N,N-dimethylaminooxyethyl]-5-methyluridine as a white foam
(14.6g, 80%).

2'-O-(dimethylaminooxyethyl)-5-methyluridine

20   Triethylamine trihydrofluoride (3.91mL, 24.0mmol) was
dissolved in dry THF and triethylamine (1.67mL, 12mmol,
dry, kept over KOH). This mixture of triethylamine-2HF was
then added to 5'-O-*tert*-butyldiphenylsilyl-2'-O-[N,N-
dimethylaminooxyethyl]-5-methyluridine (1.40g, 2.4mmol) and
25   stirred at room temperature for 24 hrs. Reaction was
monitored by TLC (5% MeOH in CH$_2$Cl$_2$). Solvent was removed
under vacuum and the residue placed on a flash column and
eluted with 10% MeOH in CH$_2$Cl$_2$ to get 2'-O-
(dimethylaminooxyethyl)-5-methyluridine (766mg, 92.5%).

30   **5'-O-DMT-2'-O-(dimethylaminooxyethyl)-5-methyluridine**
           2'-O-(dimethylaminooxyethyl)-5-methyluridine (750mg,
2.17mmol) was dried over P$_2$O$_5$ under high vacuum overnight at
40°C. It was then co-evaporated with anhydrous pyridine
(20mL). The residue obtained was dissolved in pyridine
35   (11mL) under argon atmosphere. 4-dimethylaminopyridine

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(26.5mg, 2.60mmol), 4,4'-dimethoxytrityl chloride (880mg,
2.60mmol) was added to the mixture and the reaction mixture
was stirred at room temperature until all of the starting
material disappeared. Pyridine was removed under vacuum
5   and the residue chromatographed and eluted with 10% MeOH in
CH$_2$Cl$_2$ (containing a few drops of pyridine) to get 5'-O-DMT-
2'-O-(dimethylamino-oxyethyl)-5-methyluridine (1.13g, 80%).

**5'-O-DMT-2'-O-(2-N,N-dimethylaminooxyethyl)-5-
methyluridine-3'-[(2-cyanoethyl)-N,N-
10   diisopropylphosphoramidite]**

5'-O-DMT-2'-O-(dimethylaminooxyethyl)-5-methyluridine
(1.08g, 1.67mmol) was co-evaporated with toluene (20mL).
To the residue N,N-diisopropylamine tetrazonide (0.29g,
1.67mmol) was added and dried over P$_2$O$_5$ under high vacuum
15   overnight at 40°C. Then the reaction mixture was dissolved
in anhydrous acetonitrile (8.4mL) and 2-cyanoethyl-
N,N,N^1,N^1-tetraisopropylphosphoramidite (2.12mL, 6.08mmol)
was added. The reaction mixture was stirred at ambient
temperature for 4 hrs under inert atmosphere. The progress
20   of the reaction was monitored by TLC (hexane:ethyl acetate
1:1). The solvent was evaporated, then the residue was
dissolved in ethyl acetate (70mL) and washed with 5%
aqueous NaHCO$_3$ (40mL). Ethyl acetate layer was dried over
anhydrous Na$_2$SO$_4$ and concentrated. Residue obtained was
25   chromatographed (ethyl acetate as eluent) to get 5'-O-DMT-
2'-O-(2-N,N-dimethylaminooxyethyl)-5-methyluridine-3'-[(2-
cyanoethyl)-N,N-diisopropylphosphoramidite] as a foam
(1.04g, 74.9%).

2'-(Aminooxyethoxy) nucleoside amidites

30   2'-(Aminooxyethoxy) nucleoside amidites [also known in
the art as 2'-O-(aminooxyethyl) nucleoside amidites] are
prepared as described in the following paragraphs.
Adenosine, cytidine and thymidine nucleoside amidites are
prepared similarly.

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N2-isobutyryl-6-O-diphenylcarbamoyl-2'-O-(2-ethylacetyl)-5'-O-(4,4'-dimethoxytrityl)guanosine-3'-[(2-cyanoethyl)-N,N-diisopropylphosphoramidite]

The 2'-O-aminooxyethyl guanosine analog may be
obtained by selective 2'-O-alkylation of diaminopurine
riboside. Multigram quantities of diaminopurine riboside
may be purchased from Schering AG (Berlin) to provide 2'-O-
(2-ethylacetyl) diaminopurine riboside along with a minor
amount of the 3'-O-isomer. 2'-O-(2-ethylacetyl)
diaminopurine riboside may be resolved and converted to 2'-
O-(2-ethylacetyl)guanosine by treatment with adenosine
deaminase. (McGee, D. P. C., Cook, P. D., Guinosso, C. J.,
WO 94/02501 A1 940203.) Standard protection procedures
should afford 2'-O-(2-ethylacetyl)-5'-O-(4,4'-
dimethoxytrityl)guanosine and 2-N-isobutyryl-6-O-
diphenylcarbamoyl-2'-O-(2-ethylacetyl)-5'-O-(4,4'-
dimethoxytrityl)guanosine which may be reduced to provide
2-N-isobutyryl-6-O-diphenylcarbamoyl-2'-O-(2-ethylacetyl)-
5'-O-(4,4'-dimethoxytrityl)guanosine. As before the
hydroxyl group may be displaced by N-hydroxyphthalimide via
a Mitsunobu reaction, and the protected nucleoside may
phosphitylated as usual to yield 2-N-isobutyryl-6-O-
diphenylcarbamoyl-2'-O-(2-ethylacetyl)-5'-O-(4,4'-
dimethoxytrityl)guanosine-3'-[(2-cyanoethyl)-N,N-
diisopropylphosphoramidite].

2'-dimethylaminoethoxyethoxy (2'-DMAEOE) nucleoside amidites

2'-dimethylaminoethoxyethoxy nucleoside amidites (also
known in the art as 2'-O-dimethylaminoethoxyethyl, i.e.,
2'-O-CH_2-O-CH_2-N$(CH_2)_2$, or 2'-DMAEOE nucleoside amidites) are
prepared as follows. Other nucleoside amidites are prepared
similarly.

2'-O-[2(2-N,N-dimethylaminoethoxy)ethyl]-5-methyl uridine

2[2-(Dimethylamino)ethoxy]ethanol (Aldrich, 6.66 g, 50

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mmol) is slowly added to a solution of borane in tetra-
hydrofuran (1 M, 10 mL, 10 mmol) with stirring in a 100 mL
bomb. Hydrogen gas evolves as the solid dissolves. $O^2-,2'-$
anhydro-5-methyluridine (1.2 g, 5 mmol), and sodium

5   bicarbonate (2.5 mg) are added and the bomb is sealed,
placed in an oil bath and heated to 155°C for 26 hours.
The bomb is cooled to room temperature and opened. The
crude solution is concentrated and the residue partitioned
between water (200 mL) and hexanes (200 mL). The excess

10   phenol is extracted into the hexane layer. The aqueous
layer is extracted with ethyl acetate (3x200 mL) and the
combined organic layers are washed once with water, dried
over anhydrous sodium sulfate and concentrated. The
residue is columned on silica gel using methanol/methylene

15   chloride 1:20 (which has 2% triethylamine) as the eluent.
As the column fractions are concentrated a colorless solid
forms which is collected to give the title compound as a
white solid.

5'-O-dimethoxytrityl-2'-O-[2(2-N,N-dimethylaminoethoxy)

20   **ethyl)]-5-methyl uridine**

To 0.5 g (1.3 mmol) of 2'-O-[2(2-N,N-dimethylamino-
ethoxy)ethyl)]-5-methyl uridine in anhydrous pyridine (8
mL), triethylamine (0.36 mL) and dimethoxytrityl chloride
(DMT-Cl, 0.87 g, 2 eq.) are added and stirred for 1 hour.

25   The reaction mixture is poured into water (200 mL) and
extracted with CH_2Cl_2 (2x200 mL). The combined CH_2Cl_2 layers
are washed with saturated $NaHCO_3$ solution, followed by
saturated NaCl solution and dried over anhydrous sodium
sulfate. Evaporation of the solvent followed by silica gel

30   chromatography using MeOH:CH_2Cl_2:Et_3N (20:1, v/v, with 1%
triethylamine) gives the title compound.

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5'-O-Dimethoxytrityl-2'-O-[2(2-N,N-
dimethylaminoethoxy)ethyl)]-5-methyl uridine-3'-O-
(cyanoethyl-N,N-diisopropyl)phosphoramidite

Diisopropylaminotetrazolide (0.6 g) and 2-cyanoethoxy-

5   N,N-diisopropyl phosphoramidite (1.1 mL, 2 eq.) are added
to a solution of 5'-O-dimethoxytrityl-2'-O-[2(2-N,N-
dimethylaminoethoxy)ethyl)]-5-methyluridine (2.17 g, 3
mmol) dissolved in CH$_2$Cl$_2$ (20 mL) under an atmosphere of
argon. The reaction mixture is stirred overnight and the

10   solvent evaporated. The resulting residue is purified by
silica gel flash column chromatography with ethyl acetate
as the eluent to give the title compound.

Example 2

Oligonucleotide synthesis

15   Unsubstituted and substituted phosphodiester (P=O)
oligonucleotides are synthesized on an automated DNA
synthesizer (Applied Biosystems model 380B) using standard
phosphoramidite chemistry with oxidation by iodine.

Phosphorothioates (P=S) are synthesized as for the

20   phosphodiester oligonucleotides except the standard
oxidation bottle was replaced by 0.2 M solution of 3H-1,2-
benzodithiole-3-one 1,1-dioxide in acetonitrile for the
stepwise thiation of the phosphite linkages. The thiation
wait step was increased to 68 sec and was followed by the

25   capping step. After cleavage from the CPG column and
deblocking in concentrated ammonium hydroxide at 55°C (18
h), the oligonucleotides were purified by precipitating
twice with 2.5 volumes of ethanol from a 0.5 M NaCl
solution.   Phosphinate oligonucleotides are prepared as

30   described in U.S. Patent 5,508,270, herein incorporated by
reference.

Alkyl phosphonate oligonucleotides are prepared as
described in U.S. Patent 4,469,863, herein incorporated by
reference.

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3'-Deoxy-3'-methylene phosphonate oligonucleotides are prepared as described in U.S. Patents 5,610,289 or 5,625,050, herein incorporated by reference.

Phosphoramidite oligonucleotides are prepared as described in U.S. Patent, 5,256,775 or U.S. Patent 5,366,878, herein incorporated by reference.

Alkylphosphonothioate oligonucleotides are prepared as described in published PCT applications PCT/US94/00902 and PCT/US93/06976 (published as WO 94/17093 and WO 94/02499, respectively), herein incorporated by reference.

3'-Deoxy-3'-amino phosphoramidate oligonucleotides are prepared as described in U.S. Patent 5,476,925, herein incorporated by reference.

Phosphotriester oligonucleotides are prepared as described in U.S. Patent 5,023,243, herein incorporated by reference.

Borano phosphate oligonucleotides are prepared as described in U.S. Patents 5,130,302 and 5,177,198, both herein incorporated by reference.

Example 3

Oligonucleoside Synthesis

Methylenemethylimino linked oligonucleosides, also identified as MMI linked oligonucleosides, methylenedi-methylhydrazo linked oligonucleosides, also identified as MDH linked oligonucleosides, and methylenecarbonylamino linked oligonucleosides, also identified as amide-3 linked oligonucleosides, and methyleneaminocarbonyl linked oligo-nucleosides, also identified as amide-4 linked oligonucleo-sides, as well as mixed backbone compounds having, for instance, alternating MMI and P=O or P=S linkages are prepared as described in U.S. Patents 5,378,825, 5,386,023, 5,489,677, 5,602,240 and 5,610,289, all of which are herein incorporated by reference.

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Formacetal and thioformacetal linked oligonucleosides are prepared as described in U.S. Patents 5,264,562 and 5,264,564, herein incorporated by reference.

Ethylene oxide linked oligonucleosides are prepared as
5   described in U.S. Patent 5,223,618, herein incorporated by reference.

Example 4

PNA Synthesis

Peptide nucleic acids (PNAs) are prepared in
10 accordance with any of the various procedures referred to in Peptide Nucleic Acids (PNA): Synthesis, Properties and Potential Applications, *Bioorganic & Medicinal Chemistry*, **1996**, *4*, 5-23. They may also be prepared in accordance with U.S. Patents 5,539,082, 5,700,922, and 5,719,262,
15 herein incorporated by reference.

Example 5

Synthesis of Chimeric Oligonucleotides

Chimeric oligonucleotides, oligonucleosides or mixed oligonucleotides/oligonucleosides of the invention can be
20 of several different types. These include a first type wherein the "gap" segment of linked nucleosides is positioned between 5' and 3' "wing" segments of linked nucleosides and a second "open end" type wherein the "gap" segment is located at either the 3' or the 5' terminus of
25 the oligomeric compound. Oligonucleotides of the first type are also known in the art as "gapmers" or gapped oligonucleotides. Oligonucleotides of the second type are also known in the art as "hemimers" or "wingmers".

[2'-O-Me]--[2'-deoxy]--[2'-O-Me] Chimeric
30   **Phosphorothioate Oligonucleotides**

Chimeric oligonucleotides having 2'-O-alkyl phosphorothioate and 2'-deoxy phosphorothioate oligo-nucleotide segments are synthesized using an Applied Biosystems automated DNA synthesizer Model 380B, as above.

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Oligonucleotides are synthesized using the automated
synthesizer and 2'-deoxy-5'-dimethoxytrityl-3'-O-phosphor-
amidite for the DNA portion and 5'-dimethoxytrityl-2'-O-
methyl-3'-O-phosphoramidite for 5' and 3' wings. The

5   standard synthesis cycle is modified by increasing the wait
step after the delivery of tetrazole and base to 600 s
repeated four times for RNA and twice for 2'-O-methyl. The
fully protected oligonucleotide is cleaved from the support
and the phosphate group is deprotected in 3:1

10   ammonia/ethanol at room temperature overnight then
lyophilized to dryness. Treatment in methanolic ammonia
for 24 hrs at room temperature is then done to deprotect
all bases and sample was again lyophilized to dryness. The
pellet is resuspended in 1M TBAF in THF for 24 hrs at room

15   temperature to deprotect the 2' positions. The reaction is
then quenched with 1M TEAA and the sample is then reduced
to 1/2 volume by rotovac before being desalted on a G25
size exclusion column. The oligo recovered is then
analyzed spectrophotometrically for yield and for purity by

20   capillary electrophoresis and by mass spectrometry.

[2'-O-(2-Methoxyethyl)]--[2'-deoxy]--[2'-O-
(Methoxyethyl)] Chimeric Phosphorothioate
Oligonucleotides

[2'-O-(2-methoxyethyl)]--[2'-deoxy]--[-2'-O-(methoxy-

25   ethyl)] chimeric phosphorothioate oligonucleotides were
prepared as per the procedure above for the 2'-O-methyl
chimeric oligonucleotide, with the substitution of 2'-O-
(methoxyethyl) amidites for the 2'-O-methyl amidites.

[2'-O-(2-Methoxyethyl)Phosphodiester]--[2'-deoxy

30   **Phosphorothioate]--[2'-O-(2-Methoxyethyl)**
Phosphodiester] Chimeric Oligonucleotides

[2'-O-(2-methoxyethyl phosphodiester]--[2'-deoxy phos-
phorothioate]--[2'-O-(methoxyethyl) phosphodiester]
chimeric oligonucleotides are prepared as per the above

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procedure for the 2'-O-methyl chimeric oligonucleotide with
the substitution of 2'-O-(methoxyethyl) amidites for the
2'-O-methyl amidites, oxidization with iodine to generate
the phosphodiester internucleotide linkages within the
5   wing portions of the chimeric structures and sulfurization
utilizing 3,H-1,2 benzodithiole-3-one 1,1 dioxide (Beaucage
Reagent) to generate the phosphorothioate internucleotide
linkages for the center gap.

Other chimeric oligonucleotides, chimeric oligonucleo-
10   sides and mixed chimeric oligonucleotides/oligonucleosides
are synthesized according to United States patent
5,623,065, herein incorporated by reference.

Example 6

Oligonucleotide Isolation

15   After cleavage from the controlled pore glass column
(Applied Biosystems) and deblocking in concentrated
ammonium hydroxide at 55°C for 18 hours, the
oligonucleotides or oligonucleosides are purified by
precipitation twice out of 0.5 M NaCl with 2.5 volumes
20   ethanol. Synthesized oligonucleotides were analyzed by
polyacrylamide gel electrophoresis on denaturing gels and
judged to be at least 85% full length material. The
relative amounts of phosphorothioate and phosphodiester
linkages obtained in synthesis were periodically checked by
25   ^{31}P nuclear magnetic resonance spectroscopy, and for some
studies oligonucleotides were purified by HPLC, as
described by Chiang et al., *J. Biol. Chem.* 1991, *266*,
18162-18171. Results obtained with HPLC-purified material
were similar to those obtained with non-HPLC purified
30   material.

Example 7

Oligonucleotide Synthesis - 96 Well Plate Format

Oligonucleotides were synthesized via solid phase
P(III) phosphoramidite chemistry on an automated

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synthesizer capable of assembling 96 sequences
simultaneously in a standard 96 well format.
Phosphodiester internucleotide linkages were afforded by
oxidation with aqueous iodine. Phosphorothioate
5   internucleotide linkages were generated by sulfurization
utilizing 3,H-1,2 benzodithiole-3-one 1,1 dioxide (Beaucage
Reagent) in anhydrous acetonitrile. Standard base-
protected beta-cyanoethyldiisopropyl phosphoramidites were
purchased from commercial vendors (e.g. PE-Applied
10 Biosystems, Foster City, CA, or Pharmacia, Piscataway, NJ).
Non-standard nucleosides are synthesized as per known
literature or patented methods. They are utilized as base
protected beta-cyanoethyldiisopropyl phosphoramidites.

Oligonucleotides were cleaved from support and
15 deprotected with concentrated NH_4OH at elevated temperature
(55-60°C) for 12-16 hours and the released product then
dried in vacuo. The dried product was then re-suspended in
sterile water to afford a master plate from which all
analytical and test plate samples are then diluted
20 utilizing robotic pipettors.

Example 8

Oligonucleotide Analysis - 96 Well Plate Format

The concentration of oligonucleotide in each well was
assessed by dilution of samples and UV absorption
25 spectroscopy. The full-length integrity of the individual
products was evaluated by capillary electrophoresis (CE) in
either the 96 well format (Beckman P/ACE™ MDQ) or, for
individually prepared samples, on a commercial CE apparatus
(e.g., Beckman P/ACE™ 5000, ABI 270). Base and backbone
30 composition was confirmed by mass analysis of the compounds
utilizing electrospray-mass spectroscopy. All assay test
plates were diluted from the master plate using single and
multi-channel robotic pipettors. Plates were judged to be
acceptable if at least 85% of the compounds on the plate
35 were at least 85% full length.

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Example 9

Cell culture and oligonucleotide treatment

The effect of antisense compounds on target nucleic acid expression can be tested in any of a variety of cell

5 types provided that the target nucleic acid is present at measurable levels. This can be routinely determined using, for example, PCR or Northern blot analysis. The following four cell types are provided for illustrative purposes, but other cell types can be routinely used.

10

T-24 cells:

The transitional cell bladder carcinoma cell line T-24 was obtained from the American Type Culture Collection (ATCC) (Manassas, VA). T-24 cells were routinely cultured

15 in complete McCoy's 5A basal media (Gibco/Life Technologies, Gaithersburg, MD) supplemented with 10% fetal calf serum (Gibco/Life Technologies, Gaithersburg, MD), penicillin 100 units per mL, and streptomycin 100 micrograms per mL (Gibco/Life Technologies, Gaithersburg,

20 MD). Cells were routinely passaged by trypsinization and dilution when they reached 90% confluence. Cells were seeded into 96-well plates (Falcon-Primaria #3872) at a density of 7000 cells/well for use in RT-PCR analysis.

For Northern blotting or other analysis, cells may be

25 seeded onto 100 mm or other standard tissue culture plates and treated similarly, using appropriate volumes of medium and oligonucleotide.

A549 cells:

30 The human lung carcinoma cell line A549 was obtained from the American Type Culture Collection (ATCC) (Manassas, VA). A549 cells were routinely cultured in DMEM basal media (Gibco/Life Technologies, Gaithersburg, MD) supplemented with 10% fetal calf serum (Gibco/Life

35 Technologies, Gaithersburg, MD), penicillin 100 units per

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mL, and streptomycin 100 micrograms per mL (Gibco/Life
Technologies, Gaithersburg, MD). Cells were routinely
passaged by trypsinization and dilution when they reached
90% confluence.

5

NHDF cells:

Human neonatal dermal fibroblast (NHDF) were obtained
from the Clonetics Corporation (Walkersville MD). NHDFs
were routinely maintained in Fibroblast Growth Medium
10   (Clonetics Corporation, Walkersville MD) supplemented as
recommended by the supplier. Cells were maintained for up
to 10 passages as recommended by the supplier.

HEK cells:

15   Human embryonic keratinocytes (HEK) were obtained from
the Clonetics Corporation (Walkersville MD). HEKs were
routinely maintained in Keratinocyte Growth Medium
(Clonetics Corporation, Walkersville MD) formulated as
recommended by the supplier. Cells were routinely
20   maintained for up to 10 passages as recommended by the
supplier.

Treatment with antisense compounds:

When cells reached 80% confluency, they were treated
25   with oligonucleotide. For cells grown in 96-well plates,
wells were washed once with 200 μL OPTI-MEM™-1 reduced-
serum medium (Gibco BRL) and then treated with 130 μL of
OPTI-MEM™-1 containing 3.75 μg/mL LIPOFECTIN™ (Gibco BRL)
and the desired oligonucleotide at a final concentration of
30   150 nM. After 4 hours of treatment, the medium was
replaced with fresh medium. Cells were harvested 16 hours
after oligonucleotide treatment.

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Example 10

**Analysis of oligonucleotide inhibition of G-alpha-i3
expression**

Antisense modulation of G-alpha-i3 expression can be

5   assayed in a variety of ways known in the art. For
example, G-alpha-i3 mRNA levels can be quantitated by,
e.g., Northern blot analysis, competitive polymerase chain
reaction (PCR), or real-time PCR (RT-PCR). Real-time
quantitative PCR is presently preferred. RNA analysis can

10 be performed on total cellular RNA or poly(A)+ mRNA.
Methods of RNA isolation are taught in, for example,
Ausubel, *F.M. et al., Current Protocols in Molecular
Biology*, Volume 1, pp. 4.1.1-4.2.9 and 4.5.1-4.5.3, John
Wiley & Sons, Inc., **1993**. Northern blot analysis is

15 routine in the art and is taught in, for example, Ausubel,
F.M. et al., *Current Protocols in Molecular Biology*, Volume
1, pp. 4.2.1-4.2.9, John Wiley & Sons, Inc., **1996**. Real-
time quantitative (PCR) can be conveniently accomplished
using the commercially available ABI PRISM™ 7700 Sequence

20 Detection System, available from PE-Applied Biosystems,
Foster City, CA and used according to manufacturer's
instructions. Other methods of PCR are also known in the
art.

G-alpha-i3 protein levels can be quantitated in a

25 variety of ways well known in the art, such as
immunoprecipitation, Western blot analysis
(immunoblotting), ELISA or fluorescence-activated cell
sorting (FACS). Antibodies directed to G-alpha-i3 can be
identified and obtained from a variety of sources, such as

30 the MSRS catalog of antibodies (Aerie Corporation,
Birmingham, MI), or can be prepared via conventional
antibody generation methods. Methods for preparation of
polyclonal antisera are taught in, for example, Ausubel,
F.M. et al., *Current Protocols in Molecular Biology*, Volume

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2, pp. 11.12.1-11.12.9, John Wiley & Sons, Inc., 1997.
Preparation of monoclonal antibodies is taught in, for
example, Ausubel, F.M. et al., *Current Protocols in
Molecular Biology*, Volume 2, pp. 11.4.1-11.11.5, John Wiley
5   & Sons, Inc., 1997.

Immunoprecipitation methods are standard in the art
and can be found at, for example, Ausubel, F.M. et al.,
Current Protocols in Molecular Biology, Volume 2, pp.
10.16.1-10.16.11, John Wiley & Sons, Inc., 1998. Western
10 blot (immunoblot) analysis is standard in the art and can
be found at, for example, Ausubel, F.M. et al., *Current
Protocols in Molecular Biology*, Volume 2, pp. 10.8.1-
10.8.21, John Wiley & Sons, Inc., 1997. Enzyme-linked
immunosorbent assays (ELISA) are standard in the art and
15 can be found at, for example, Ausubel, F.M. et al., *Current
Protocols in Molecular Biology*, Volume 2, pp. 11.2.1-
11.2.22, John Wiley & Sons, Inc., 1991.

Example 11

Poly(A)+ mRNA isolation

20   Poly(A)+ mRNA was isolated according to Miura et al.,
Clin. Chem., 1996, *42*, 1758-1764. Other methods for
poly(A)+ mRNA isolation are taught in, for example,
Ausubel, F.M. et al., *Current Protocols in Molecular
Biology*, Volume 1, pp. 4.5.1-4.5.3, John Wiley & Sons,
25 Inc., 1993. Briefly, for cells grown on 96-well plates,
growth medium was removed from the cells and each well was
washed with 200 µL cold PBS. 60 µL lysis buffer (10 mM
Tris-HCl, pH 7.6, 1 mM EDTA, 0.5 M NaCl, 0.5% NP-40, 20 mM
vanadyl-ribonucleoside complex) was added to each well, the
30 plate was gently agitated and then incubated at room
temperature for five minutes. 55 µL of lysate was
transferred to Oligo d(T) coated 96-well plates (AGCT Inc.,
Irvine CA). Plates were incubated for 60 minutes at room
temperature, washed 3 times with 200 µL of wash buffer (10

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mM Tris-HCl pH 7.6, 1 mM EDTA, 0.3 M NaCl). After the
final wash, the plate was blotted on paper towels to remove
excess wash buffer and then air-dried for 5 minutes. 60 µL
of elution buffer (5 mM Tris-HCl pH 7.6), preheated to 70°C

5   was added to each well, the plate was incubated on a 90°C
hot plate for 5 minutes, and the eluate was then
transferred to a fresh 96-well plate.

        Cells grown on 100 mm or other standard plates may be
treated similarly, using appropriate volumes of all

10   solutions.

Example 12

Total RNA Isolation

        Total mRNA was isolated using an RNEASY 96™ kit and
buffers purchased from Qiagen Inc. (Valencia CA) following

15   the manufacturer's recommended procedures. Briefly, for
cells grown on 96-well plates, growth medium was removed
from the cells and each well was washed with 200 µL cold
PBS. 100 µL Buffer RLT was added to each well and the plate
vigorously agitated for 20 seconds. 100 µL of 70% ethanol

20   was then added to each well and the contents mixed by
pipetting three times up and down. The samples were then
transferred to the RNEASY 96™ well plate attached to a
QIAVAC™ manifold fitted with a waste collection tray and
attached to a vacuum source. Vacuum was applied for 15

25   seconds. 1 mL of Buffer RW1 was added to each well of the
RNEASY 96™ plate and the vacuum again applied for 15
seconds. 1 mL of Buffer RPE was then added to each well of
the RNEASY 96™ plate and the vacuum applied for a period of
15 seconds. The Buffer RPE wash was then repeated and the

30   vacuum was applied for an additional 10 minutes. The plate
was then removed from the QIAVAC™ manifold and blotted dry
on paper towels. The plate was then re-attached to the
QIAVAC™ manifold fitted with a collection tube rack
containing 1.2 mL collection tubes. RNA was then eluted by

35   pipetting 60 µL water into each well, incubating 1 minute,

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and then applying the vacuum for 30 seconds. The elution
step was repeated with an additional 60 µL water.

        The repetitive pipetting and elution steps may be
automated using a QIAGEN Bio-Robot 9604 (Qiagen, Inc.,
5   Valencia CA). Essentially after lysing of the cells on the
culture plate, the plate is transferred to the robot deck
where the pipetting, DNase treatment and elution steps are
carried out.

Example 13

10 **Real-time Quantitative PCR Analysis of G-alpha-i3 mRNA
Levels**

        Quantitation of G-alpha-i3 mRNA levels was determined
by real-time quantitative PCR using the ABI PRISM™ 7700
Sequence Detection System (PE-Applied Biosystems, Foster
15 City, CA) according to manufacturer's instructions. This
is a closed-tube, non-gel-based, fluorescence detection
system which allows high-throughput quantitation of
polymerase chain reaction (PCR) products in real-time. As
opposed to standard PCR, in which amplification products
20 are quantitated after the PCR is completed, products in
real-time quantitative PCR are quantitated as they
accumulate. This is accomplished by including in the PCR
reaction an oligonucleotide probe that anneals specifically
between the forward and reverse PCR primers, and contains
25 two fluorescent dyes. A reporter dye (e.g., JOE or FAM,
obtained from either Operon Technologies Inc., Alameda, CA
or PE-Applied Biosystems, Foster City, CA) is attached to
the 5' end of the probe and a quencher dye (e.g., TAMRA,
obtained from either Operon Technologies Inc., Alameda, CA
30 or PE-Applied Biosystems, Foster City, CA) is attached to
the 3' end of the probe. When the probe and dyes are
intact, reporter dye emission is quenched by the proximity
of the 3' quencher dye. During amplification, annealing of
the probe to the target sequence creates a substrate that
35 can be cleaved by the 5'-exonuclease activity of Taq

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polymerase. During the extension phase of the PCR
amplification cycle, cleavage of the probe by Taq
polymerase releases the reporter dye from the remainder of
the probe (and hence from the quencher moiety) and a
5   sequence-specific fluorescent signal is generated. With
each cycle, additional reporter dye molecules are cleaved
from their respective probes, and the fluorescence
intensity is monitored at regular intervals by laser optics
built into the ABI PRISM™ 7700 Sequence Detection System.
10   In each assay, a series of parallel reactions containing
serial dilutions of mRNA from untreated control samples
generates a standard curve that is used to quantitate the
percent inhibition after antisense oligonucleotide
treatment of test samples.

15   PCR reagents were obtained from PE-Applied Biosystems,
Foster City, CA. RT-PCR reactions were carried out by
adding 25 μL PCR cocktail (1x TAQMAN™ buffer A, 5.5 mM
MgCl$_2$, 300 μM each of dATP, dCTP and dGTP, 600 μM of dUTP,
100 nM each of forward primer, reverse primer, and probe,
20   20 Units RNAse inhibitor, 1.25 Units AMPLITAQ GOLD™, and
12.5 Units MuLV reverse transcriptase) to 96 well plates
containing 25 μL poly(A) mRNA solution. The RT reaction
was carried out by incubation for 30 minutes at 48°C.
Following a 10 minute incubation at 95°C to activate the
25   AMPLITAQ GOLD™, 40 cycles of a two-step PCR protocol were
carried out: 95°C for 15 seconds (denaturation) followed by
60°C for 1.5 minutes (annealing/extension). G-alpha-i3
probes and primers were designed to hybridize to the human
G-alpha-i3 sequence, using published sequence information
30   (GenBank accession number J03005, incorporated herein as
SEQ ID NO:1).

        For G-alpha-i3 the PCR primers were:
forward primer: GGACGGCTAAAGATTGACTTTGG (SEQ ID NO: 2)
reverse primer: CAGCACTGCCAGCTAAAACAAAT (SEQ ID NO: 3) and
35   the PCR probe was: FAM-AGGGCAGATGATGCCCGGCAAT-TAMRA

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(SEQ ID NO: 4) where FAM (PE-Applied Biosystems, Foster
City, CA) is the fluorescent reporter dye) and TAMRA (PE-
Applied Biosystems, Foster City, CA) is the quencher dye.

For GAPDH the PCR primers were:

5   forward primer: GAAGGTGAAGGTCGGAGTC (SEQ ID NO: 5)
reverse primer: GAAGATGGTGATGGGATTTC (SEQ ID NO: 6)and the
PCR probe was: 5' JOE-CAAGCTTCCCGTTCTCAGCC- TAMRA 3' (SEQ
ID NO: 7) where JOE (PE-Applied Biosystems, Foster City,
CA) is the fluorescent reporter dye) and TAMRA (PE-Applied

10   Biosystems, Foster City, CA) is the quencher dye.

Example 14

Northern blot analysis of G-alpha-i3 mRNA levels

Eighteen hours after antisense treatment, cell
monolayers were washed twice with cold PBS and lysed in 1

15   mL RNAZOL™ (TEL-TEST "B" Inc., Friendswood, TX). Total RNA
was prepared following manufacturer's recommended
protocols. Twenty micrograms of total RNA was fractionated
by electrophoresis through 1.2% agarose gels containing
1.1% formaldehyde using a MOPS buffer system (AMRESCO, Inc.

20   Solon, OH). RNA was transferred from the gel to HYBOND™-N+
nylon membranes (Amersham Pharmacia Biotech, Piscataway,
NJ) by overnight capillary transfer using a
Northern/Southern Transfer buffer system (TEL-TEST "B"
Inc., Friendswood, TX). RNA transfer was confirmed by UV

25   visualization. Membranes were fixed by UV cross-linking
using a STRATALINKER™ UV Crosslinker 2400 (Stratagene,
Inc, La Jolla, CA).

Membranes were probed using QUICKHYB™ hybridization
solution (Stratagene, La Jolla, CA) using manufacturer's

30   recommendations for stringent conditions with a G-alpha-i3
specific probe prepared by PCR using the forward primer
GGACGGCTAAAGATTGACTTTGG (SEQ ID NO: 2) and the reverse
primer CAGCACTGCCAGCTAAAACAAAT (SEQ ID NO: 3). To
normalize for variations in loading and transfer efficiency

35   membranes were stripped and probed for glyceraldehyde-3-

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phosphate dehydrogenase (GAPDH) RNA (Clontech, Palo Alto,
CA). Hybridized membranes were visualized and quantitated
using a PHOSPHORIMAGER™ and IMAGEQUANT™ Software V3.3
(Molecular Dynamics, Sunnyvale, CA). Data was normalized
5   to GAPDH levels in untreated controls.

Example 15

Antisense inhibition of G-alpha-i3 expression-
phosphorothioate oligodeoxynucleotides

In accordance with the present invention, a series of
10 oligonucleotides were designed to target different regions
of the human G-alpha-i3 RNA, using published sequences
(GenBank accession number J03005, incorporated herein as
SEQ ID NO: 1). The oligonucleotides are shown in Table 1.
Target sites are indicated by nucleotide numbers, as given
15 in the sequence source reference (Genbank accession no.
J03005), to which the oligonucleotide binds. All compounds
in Table 1 are oligodeoxynucleotides with phosphorothioate
backbones (internucleoside linkages) throughout. The
compounds were analyzed for effect on G-alpha-i3 mRNA
20 levels by quantitative real-time PCR as described in other
examples herein. Data are averages from two experiments.
If present, "N.D." indicates "no data".

Table 1

25   Inhibition of G-alpha-i3 mRNA levels by phosphorothioate
oligodeoxynucleotides

ISIS#	REGION	TARGET SITE	SEQUENCE	% Inhibition	SEQ ID NO.
25888	Coding	126	atcttgcttcgctccact	37	8
25889	Coding	278	attgtttacattcatcct	0	9
25890	Coding	353	caatctttagccgtccca	51	10
25891	Coding	427	gactccttcttcagcact	52	11
25892	Coding	477	ccaccatctcgccataac	23	12
25893	Coding	495	aatgtagttagactggga	54	13
25895	Coding	511	catcttgctgagttggaa	66	14
25896	Coding	529	tcactctcgtccgaagaa	59	15
25897	Coding	535	tggtcttcactctcgtcc	79	16

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	25898	Coding	545	acaatgcctgtggtcttc	0	17
	25899	Coding	553	gtgtttctacaatgcctg	63	18
	25900	Coding	557	aaatgtgtttctacaatg	10	19
	25894	Coding	584	gagttggaatgtagttag	44	20
5	25903	Coding	696	agccagaacaaggtcata	12	21
	25901	Coding	737	taattgctgtcactccct	35	22
	25902	Coding	768	aggtcataatcactgagg	25	23
	25904	Coding	786	aatgattgaagtttctgt	27	24
	25906	Coding	797	ttaaggaagagaatgatt	0	25
10	25907	Coding	840	taacggactcctctttat	75	26
	25905	Coding	872	agagaatgattgaagttt	1	27
	25909	Coding	939	ggtatcttttcttctgtt	52	28
	25910	Coding	947	atctccttggtatctttt	67	29
	25908	Coding	956	tattggaacctgtgtatt	46	30
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	25912	Stop Codon	1055	caataaagtccacattcc	0	32
	25914	Stop Codon	1065	ccatgcttctcaataaag	42	33
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	25918	3' UTR	1113	aaggtgtctggtctcaac	44	35
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	25924	3' UTR	1308	ttaagaaatccaagtgat	0	44
	25925	3' UTR	1340	tcaacttcacatcttcca	38	45
30	25926	3' UTR	1350	atgcagcacctcaacttc	60	46
	25927	3' UTR	1448	taaagttattcttctctt	12	47

As shown in Table 1, SEQ ID NOs 8, 10, 11, 13, 14,

15, 16, 18, 20, 22, 26, 28, 29, 30, 31, 33, 35, 37, 38, 39,

35   42, 43, 45 and 46 demonstrated at least 30% inhibition of

G-alpha-i3 expression in this assay and are therefore

preferred.

Example 16:

Antisense inhibition of G-alpha-i3 expression-

40   **phosphorothioate 2'-MOE gapmer oligonucleotides**

In accordance with the present invention, a second

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series of oligonucleotides targeted to human G-alpha-i3
were synthesized. The oligonucleotide sequences are shown
in Table 2. Target sites are indicated by nucleotide
numbers, as given in the sequence source reference (Genbank
5   accession no. J03005), to which the oligonucleotide binds.

     All compounds in Table 2 are chimeric oligonucleotides
("gapmers") 18 nucleotides in length, composed of a central
"gap" region consisting of ten 2'-deoxynucleotides, which
is flanked on both sides (5' and 3' directions) by four-
10   nucleotide "wings". The wings are composed of 2'-
methoxyethyl (2'-MOE)nucleotides. The internucleoside
(backbone) linkages are phosphorothioate (P=S) throughout
the oligonucleotide. Cytidine residues in the 2'-MOE wings
are 5-methylcytidines.

15   Data were obtained by real-time quantitative PCR as
described in other examples herein and are averaged from
two experiments. If present, "N.D." indicates "no data".

Table 2
20   Inhibition of G-alpha-i3 mRNA levels by chimeric
phosphorothioate oligonucleotides having 2'-MOE wings and a
deoxy gap

ISIS#	REGION	TARGET SITE	SEQUENCE	% Inhibition	SEQ ID NO.
25928	Coding	126	atcttgcttcgctccact	58	8
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25930	Coding	353	caatctttagccgtccca	40	10
25931	Coding	427	gactccttcttcagcact	41	11
25932	Coding	477	ccaccatctcgccataac	0	12
25933	Coding	495	aatgtagttagactggga	0	13
25935	Coding	511	catcttgctgagttggaa	40	14
25936	Coding	529	tcactctcgtccgaagaa	0	15
25937	Coding	535	tggtcttcactctcgtcc	72	16
25938	Coding	545	acaatgcctgtggtcttc	0	17
25939	Coding	553	gtgtttctacaatgcctg	41	18
25940	Coding	557	aaatgtgtttctacaatg	42	19
25934	Coding	584	gagttggaatgtagttag	39	20
25943	Coding	696	agccagaacaaggtcata	29	21
25941	Coding	737	taattgctgtcactccct	19	22

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25942	Coding	768	aggtcataatcactgagg	34	23
25944	Coding	786	aatgattgaagtttctgt	62	24
25946	Coding	797	ttaaggaagagaatgatt	21	25
25947	Coding	840	taacggactcctctttat	55	26
25945	Coding	872	agagaatgattgaagttt	20	27
25949	Coding	939	ggtatcttttcttctgtt	81	28
25950	Coding	947	atctccttggtatctttt	46	29
25948	Coding	956	tattggaacctgtgtatt	54	30
25951	Coding	1052	tggcacaggtgaagtgag	41	31
25952	Stop Codon	1055	caataaagtccacattcc	75	32
25954	Stop Codon	1065	ccatgcttctcaataaag	50	33
25957	3' UTR	1101	ctcaacactccacactgt	42	34
25958	3' UTR	1113	aaggtgtctggtctcaac	0	35
25959	3' UTR	1126	catgagacagcaaaaggt	34	36
25953	3' UTR	1142	cttctcaataaagtccac	0	37
25955	3' UTR	1172	acactgtagtaactttca	0	38
25956	3' UTR	1177	actccacactgtagtaac	71	39
25960	3' UTR	1191	taaagagtgctaagattc	18	40
25961	3' UTR	1217	aagttccctaatacaaaa	46	41
25962	3' UTR	1246	gtctgactttagcatctc	83	42
25963	3' UTR	1255	aattccaatgtctgactt	78	43
25964	3' UTR	1308	ttaagaaatccaagtgat	41	44
25965	3' UTR	1340	tcaacttcacatcttcca	35	45
25966	3' UTR	1350	atgcagcacctcaacttc	66	46
25967	3' UTR	1448	taaagttattcttctctt	47	47

As shown in Table 2, SEQ ID NOs 8, 10, 11, 14, 16, 18, 19, 20, 23, 24, 26, 28, 29, 30, 31, 32, 33, 34, 36, 39, 41, 42, 43, 44, 45, 46 and 47 demonstrated at least 30% inhibition of G-alpha-i3 expression in this experiment and are therefore preferred.

Example 17

Western blot analysis of G-alpha-i3 protein levels

Western blot analysis (immunoblot analysis) is carried out using standard methods. Cells are harvested 16-20 h after oligonucleotide treatment, washed once with PBS, suspended in Laemmli buffer (100 ul/well), boiled for 5 minutes and loaded on a 16% SDS-PAGE gel. Gels are run for 1.5 hours at 150 V, and transferred to membrane for western

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blotting. Appropriate primary antibody directed to G-
alpha-i3 is used, with a radiolabelled or fluorescently
labeled secondary antibody directed against the primary
antibody species. Bands are visualized using a
5   PHOSPHORIMAGER™ (Molecular Dynamics, Sunnyvale CA).

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What is claimed is:

1.   An antisense compound 8 to 30 nucleobases in
length targeted to a nucleic acid molecule encoding human
5   G-alpha-i3, wherein said antisense compound specifically
hybridizes with and inhibits the expression of human G-
alpha-i3.

2.   The antisense compound of claim 1 which is an
antisense oligonucleotide.
10   3.   The antisense compound of claim 2 wherein the
antisense oligonucleotide has a sequence comprising SEQ ID
NO: 8, 10, 11, 13, 20, 14, 15, 16, 18, 22, 26, 30, 28, 29,
31, 37, 33, 38, 39, 35, 42, 43, 45, 46, 19, 23, 24, 32, 34,
36, 41, 44 or 47.
15   4.   The antisense compound of claim 2 wherein the
antisense oligonucleotide has a sequence comprising SEQ ID
NO: 8, 10, 11, 20, 14, 16, 18, 26, 30, 28, 29, 31, 33, 39,
42, 43, 45 or 46.

5.   The antisense compound of claim 2 wherein the
20   antisense oligonucleotide comprises at least one modified
internucleoside linkage.

6.   The antisense compound of claim 5 wherein the
modified internucleoside linkage is a phosphorothioate
linkage.
25   7.   The antisense compound of claim 2 wherein the
antisense oligonucleotide comprises at least one modified
sugar moiety.

8.   The antisense compound of claim 7 wherein the
modified sugar moiety is a 2'-O-methoxyethyl sugar moiety.
30   9.   The antisense compound of claim 2 wherein the
antisense oligonucleotide comprises at least one modified
nucleobase.

10.   The antisense compound of claim 9 wherein the
modified nucleobase is a 5-methylcytosine.
35   11.   The antisense compound of claim 2 wherein the

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antisense oligonucleotide is a chimeric oligonucleotide.

12. A composition comprising the antisense compound of claim 1 and a pharmaceutically acceptable carrier or diluent.

5   13. The composition of claim 12 further comprising a colloidal dispersion system.

14. The composition of claim 12 wherein the antisense compound is an antisense oligonucleotide.

15. A method of inhibiting the expression of G-

10   alpha-i3 in human cells or tissues comprising contacting said cells or tissues with the antisense compound of claim 1 so that expression of G-alpha-i3 is inhibited.

16. A method of treating a human having a disease or condition associated with G-alpha-i3 comprising

15   administering to said animal a therapeutically or prophylactically effective amount of the antisense compound of claim 1 so that expression of G-alpha-i3 is inhibited.

17. The method of claim 16 wherein the disease or condition is a hyperproliferative disorder.

20   18. The method of claim 17 wherein the hyperproliferative disorder is cancer.

SEQUENCE LISTING

<110> Lex M. Cowsert
ISIS PHARMACEUTICALS, INC.

<120> ANTISENSE MODULATION OF G-ALPHA-I3 EXPRESSION

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<151> 1999-06-25

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Ala Ala Lys Glu Val Lys Leu Leu Leu Leu Gly Ala Gly Glu Ser Gly
30   35   40   45

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Arg Ser Arg Glu Tyr Gln Leu Asn Asp Ser Ala Ser Tyr Tyr Leu Asn
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A. CLASSIFICATION OF SUBJECT MATTER

IPC(7) :C07H 21/04; A61K 48/00; C12Q 1/68

US CL :435/6; 536/23.1, 24.5; 514/44

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 435/6; 536/23.1, 24.5; 514/44

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

NONE

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

EAST, MEDLINE, EMBASE, CAPLUS, SCISEARCH

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	BRANCH, A.D. A good antisense is hard to find. TIBS. February 1998,Vol. 23, pages 45-50, see entire document.	1-18
A,P	STEIN, C.A. Hybridization prediction gets to first base. Nat. Biotech. August 1999, Vol. 17, pages 751-752, see entire document.	1-18
A	GEWIRTZ et al., Facilitating oligonucleotide delivery: Helping antisense deliver on its promise. Proc. Acad. Natl. Sci. April 1996, Vol. 93, pages 3161-3163, see entire document.	1-18

☐ Further documents are listed in the continuation of Box C.   ☐ See patent family annex.

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25 FEBRUARY 2000	2 1 MAR 2000
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